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**Sibinga et al.**

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- (54) **INTRACELLULAR DOMAIN OF A MAMMALIAN FAT1 (FAT1IC)**
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- (\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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- (22) Filed: **Apr. 25, 2008**
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**Related U.S. Application Data**

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- (51) **Int. Cl.**  
*A61K 38/16* (2006.01)  
*C07K 14/00* (2006.01)
- (52) **U.S. Cl.**  
USPC ..... **514/12; 530/350**
- (58) **Field of Classification Search**  
None  
See application file for complete search history.

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(57) **ABSTRACT**

Provided is an extracellular domain of a mammalian Fat1 (Fat1<sub>EC</sub>). Also provided is an intracellular domain of a mammalian Fat1 (Fat1<sub>IC</sub>). Additionally provided is a vector comprising a nucleic acid sequence encoding the Fat1<sub>EC</sub>. A vector comprising a nucleic acid sequence encoding the Fat1<sub>IC</sub> is further provided. Also, a vascular stent coated with the Fat1<sub>EC</sub> is provided. Further provided is a vascular stent coated with a Fat1 ligand that activates Fat1. A method of treating a patient at risk for restenosis of a blood vessel is additionally provided. Further, methods of treating an injured blood vessel in a patient is provided. A method of treating a patient at risk for restenosis of a blood vessel or having an injured blood vessel is also provided.

**5 Claims, 10 Drawing Sheets**

FIG. 1

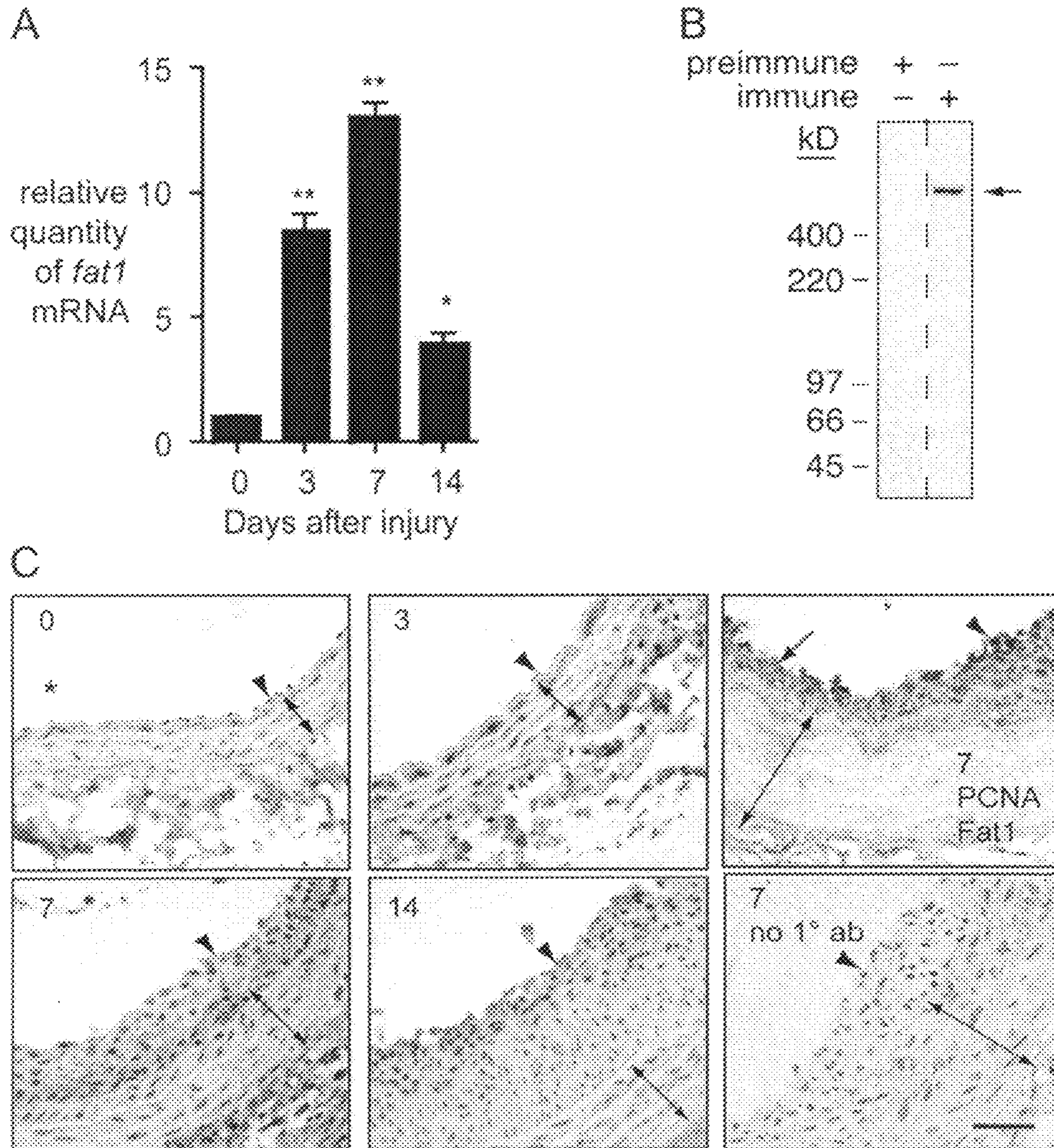


FIG. 2

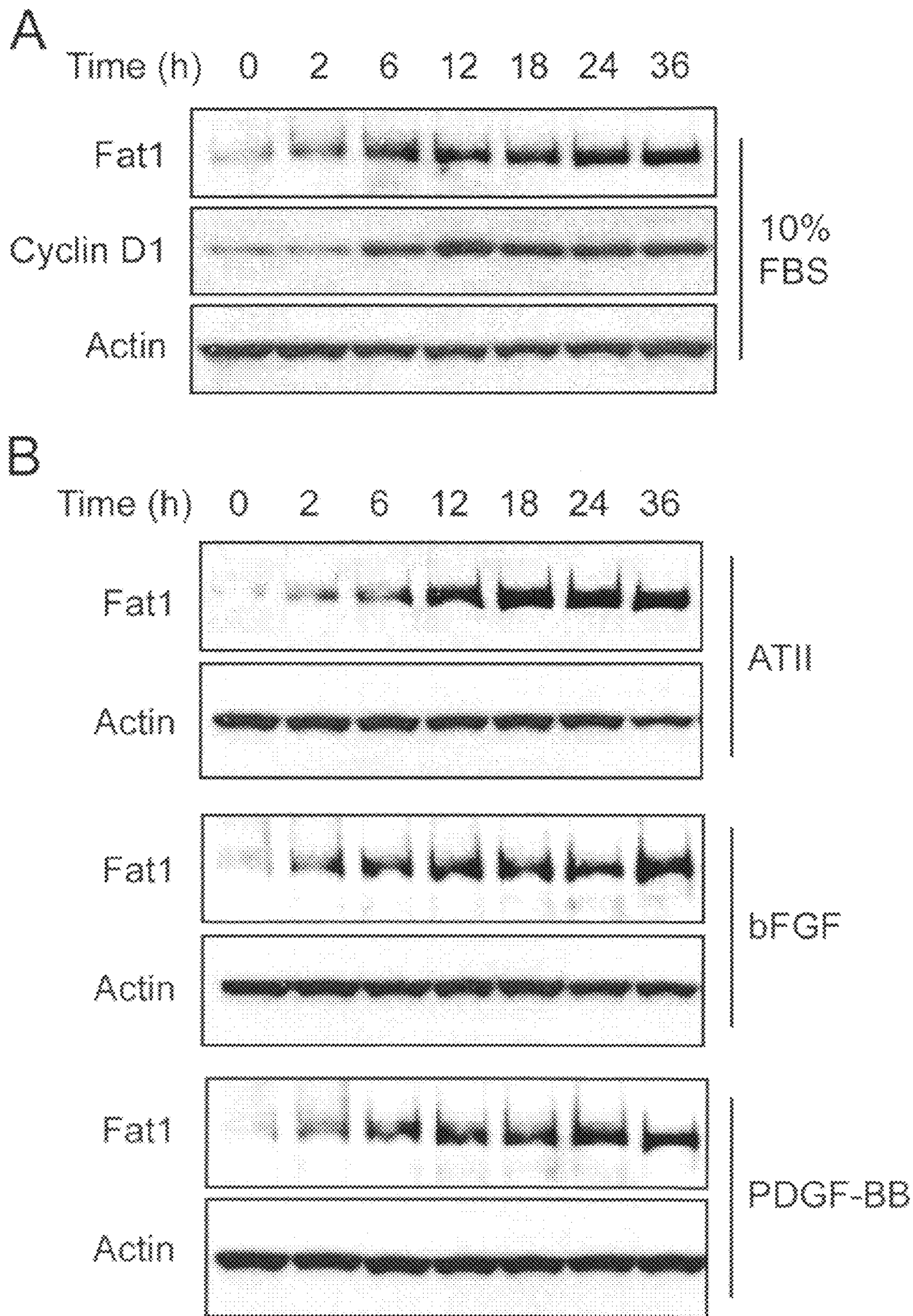
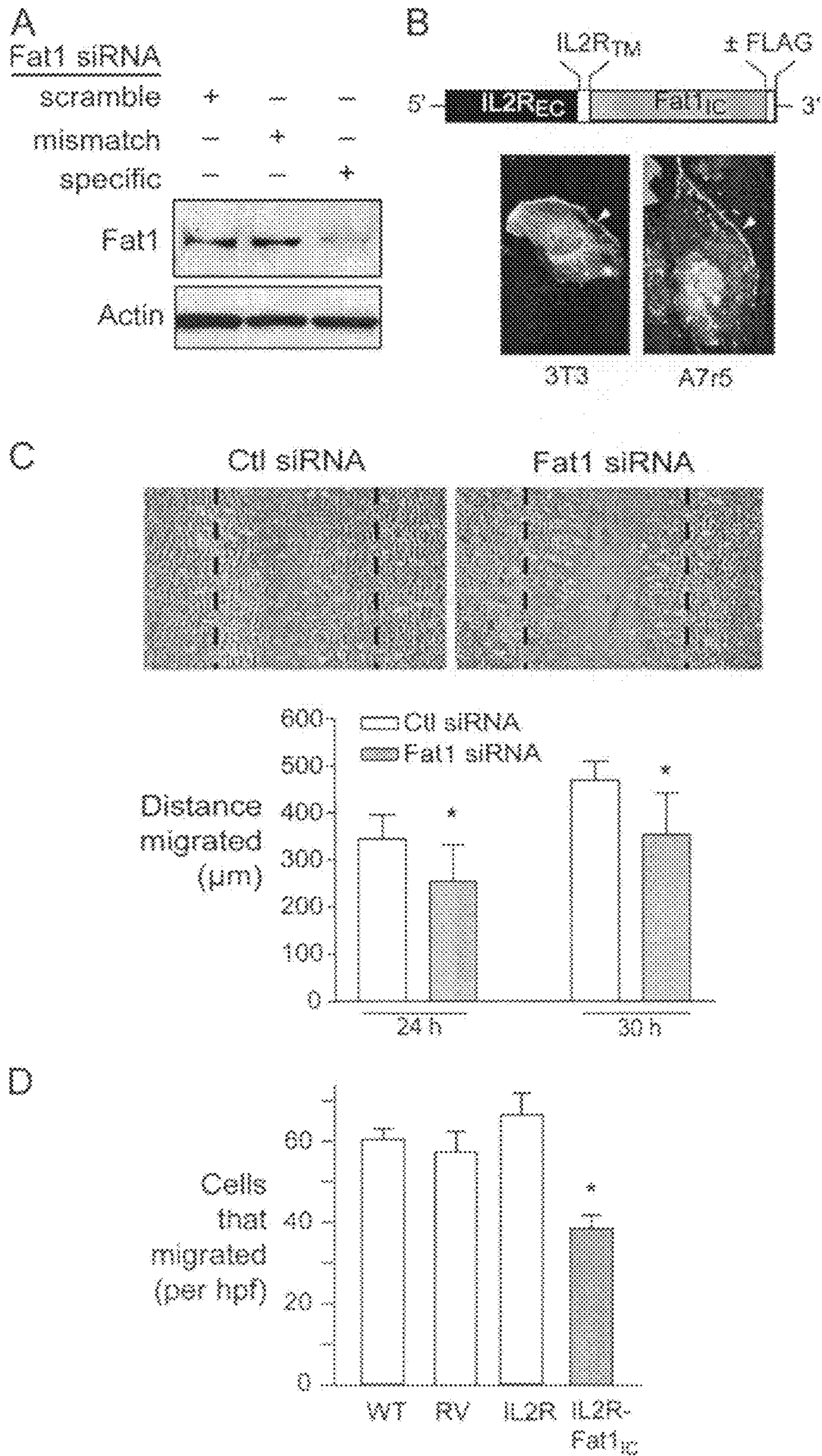


FIG. 3



**FIG. 4**

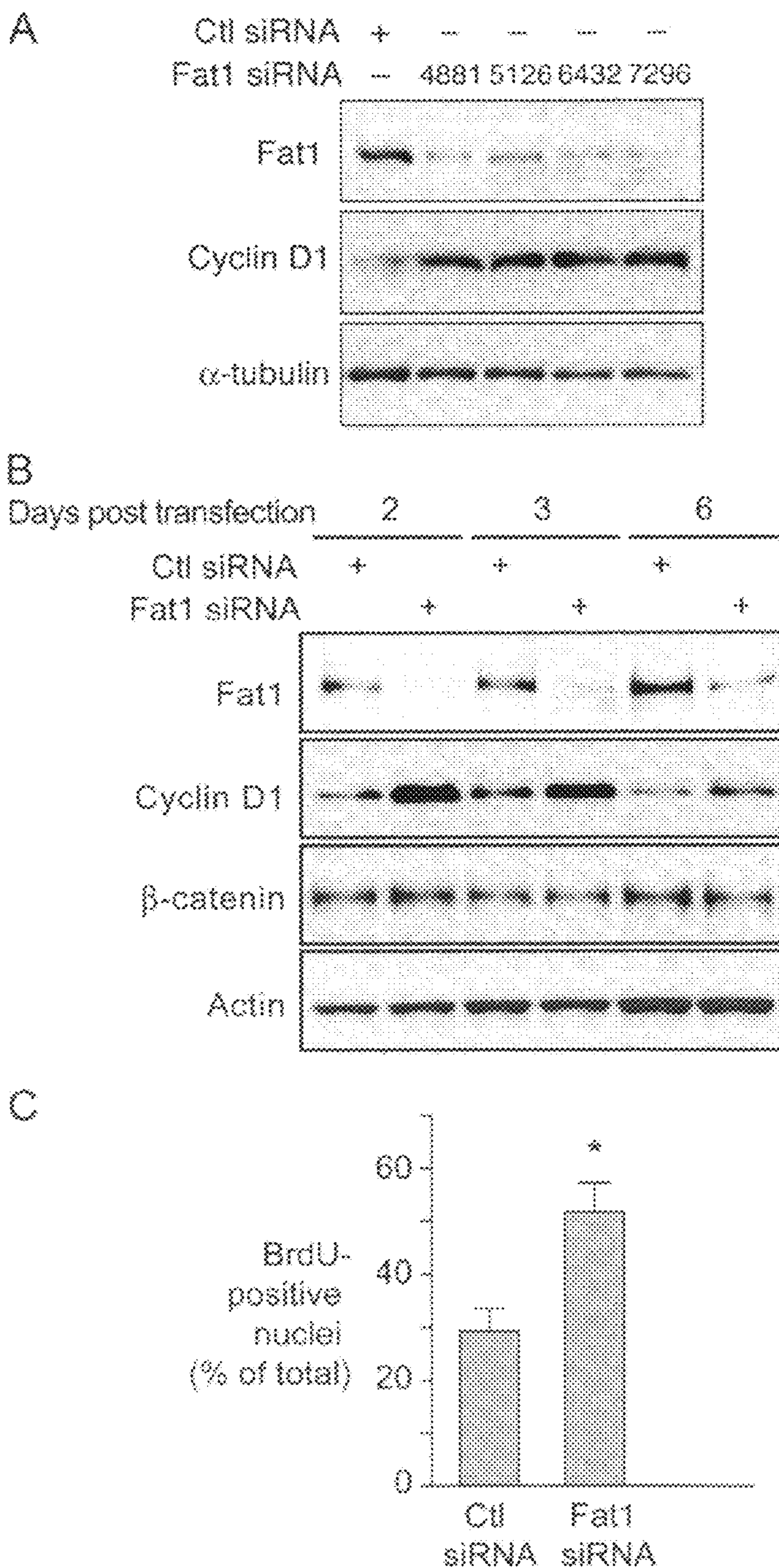


FIG. 5

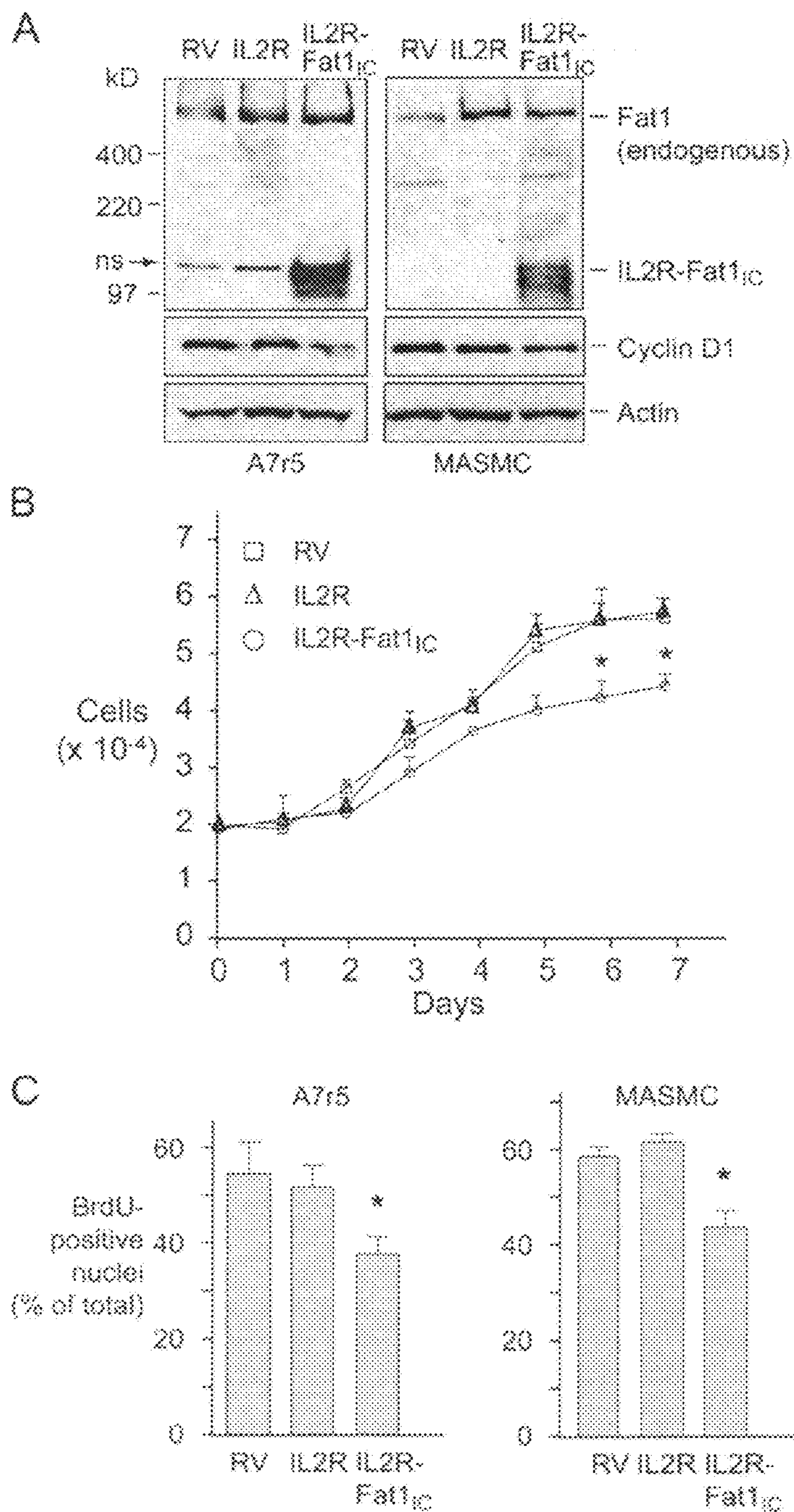


FIG. 6

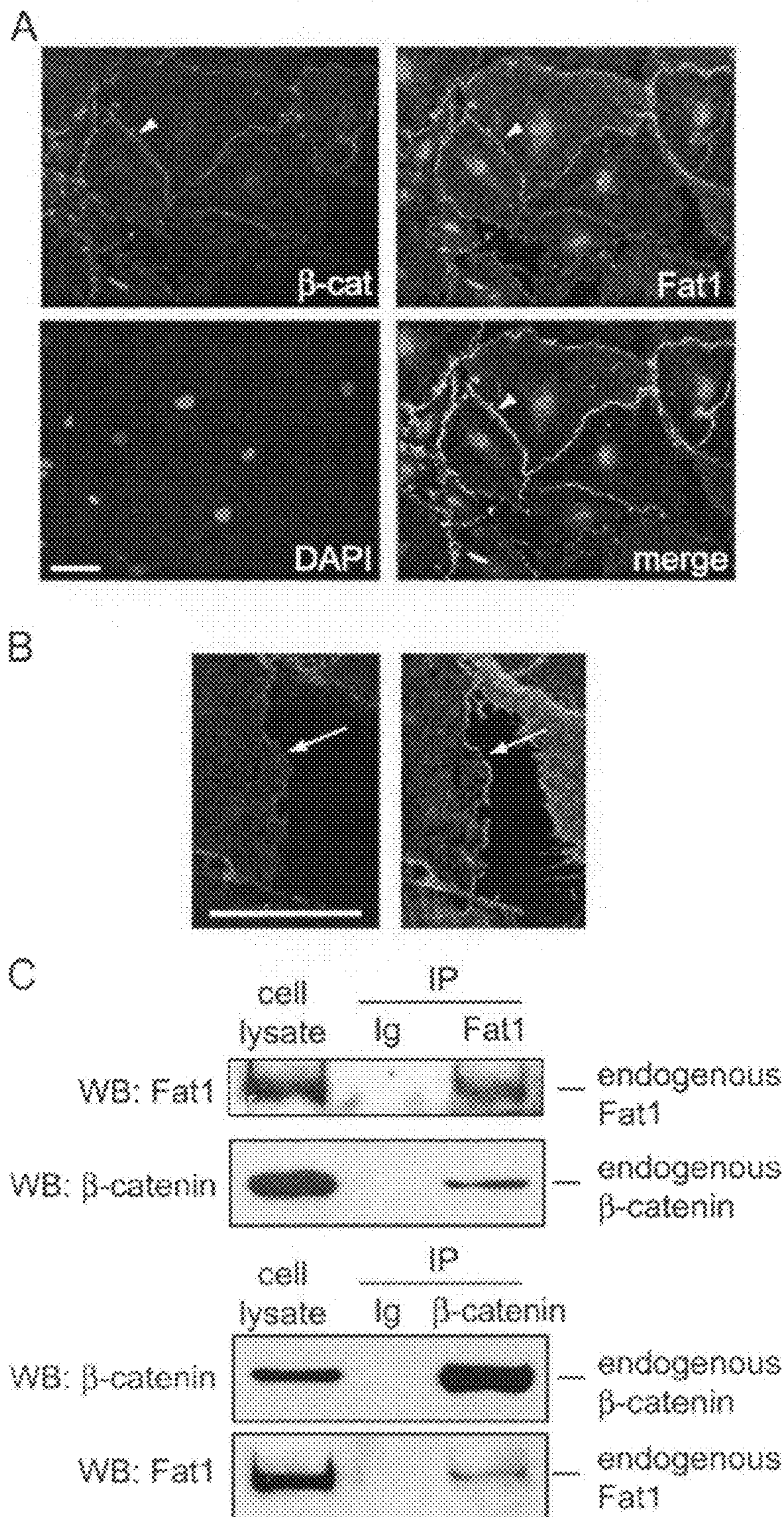


FIG. 7

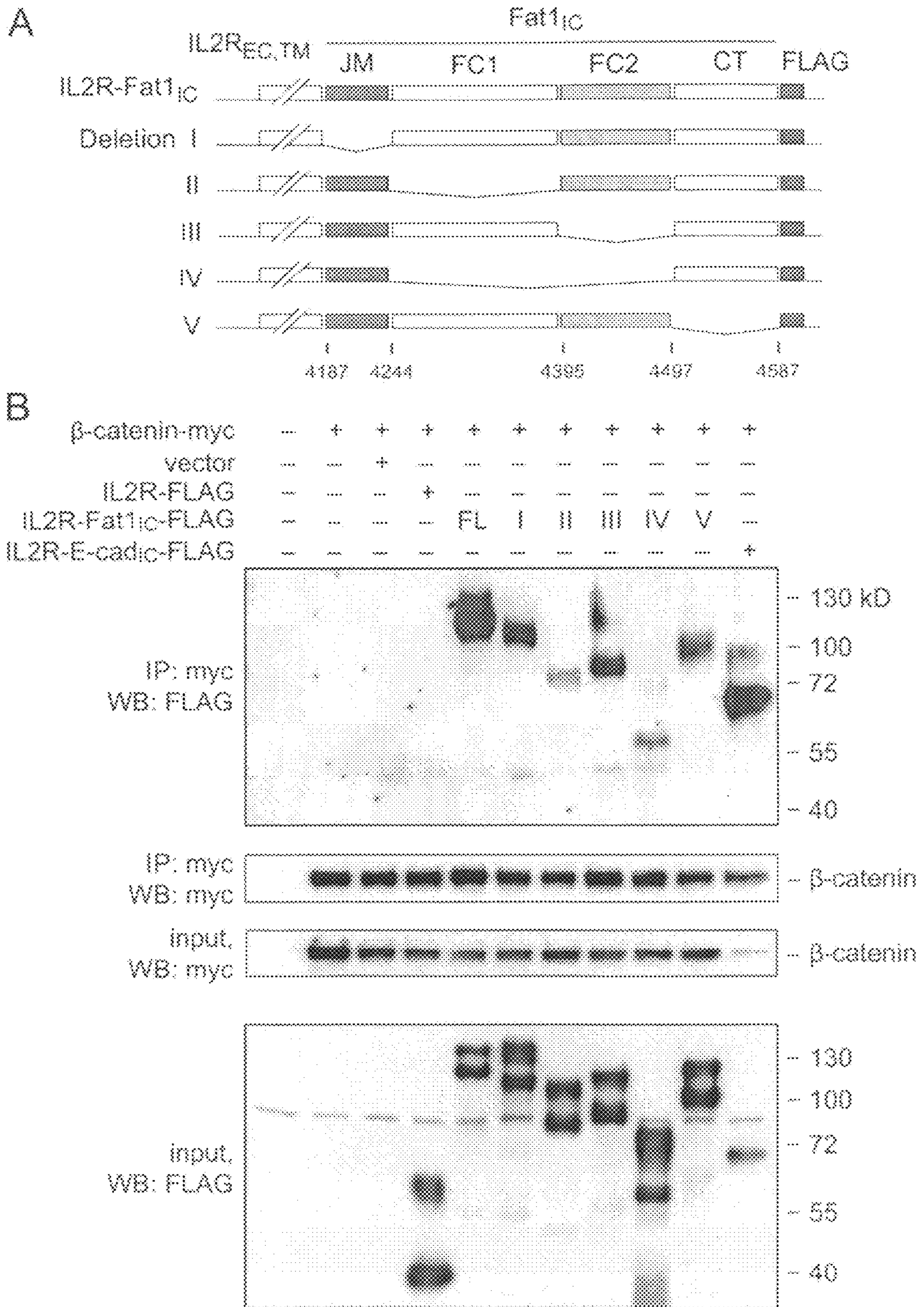




FIG. 8

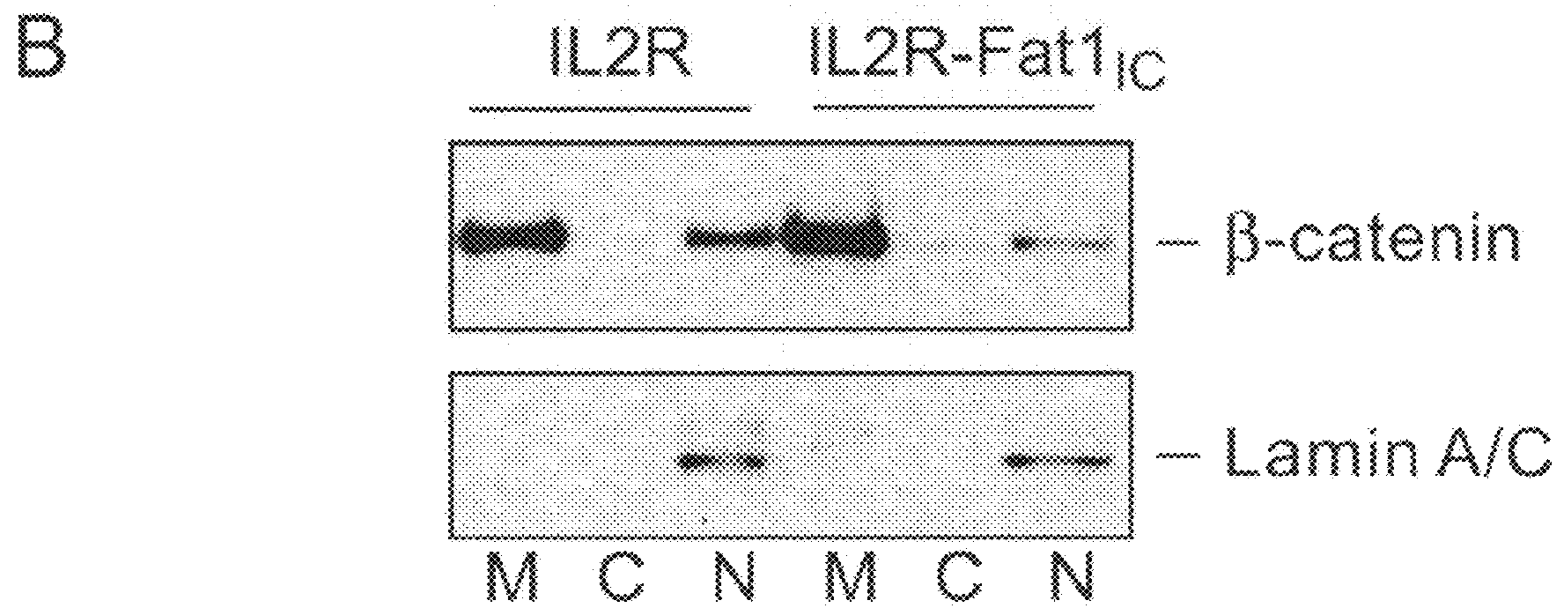
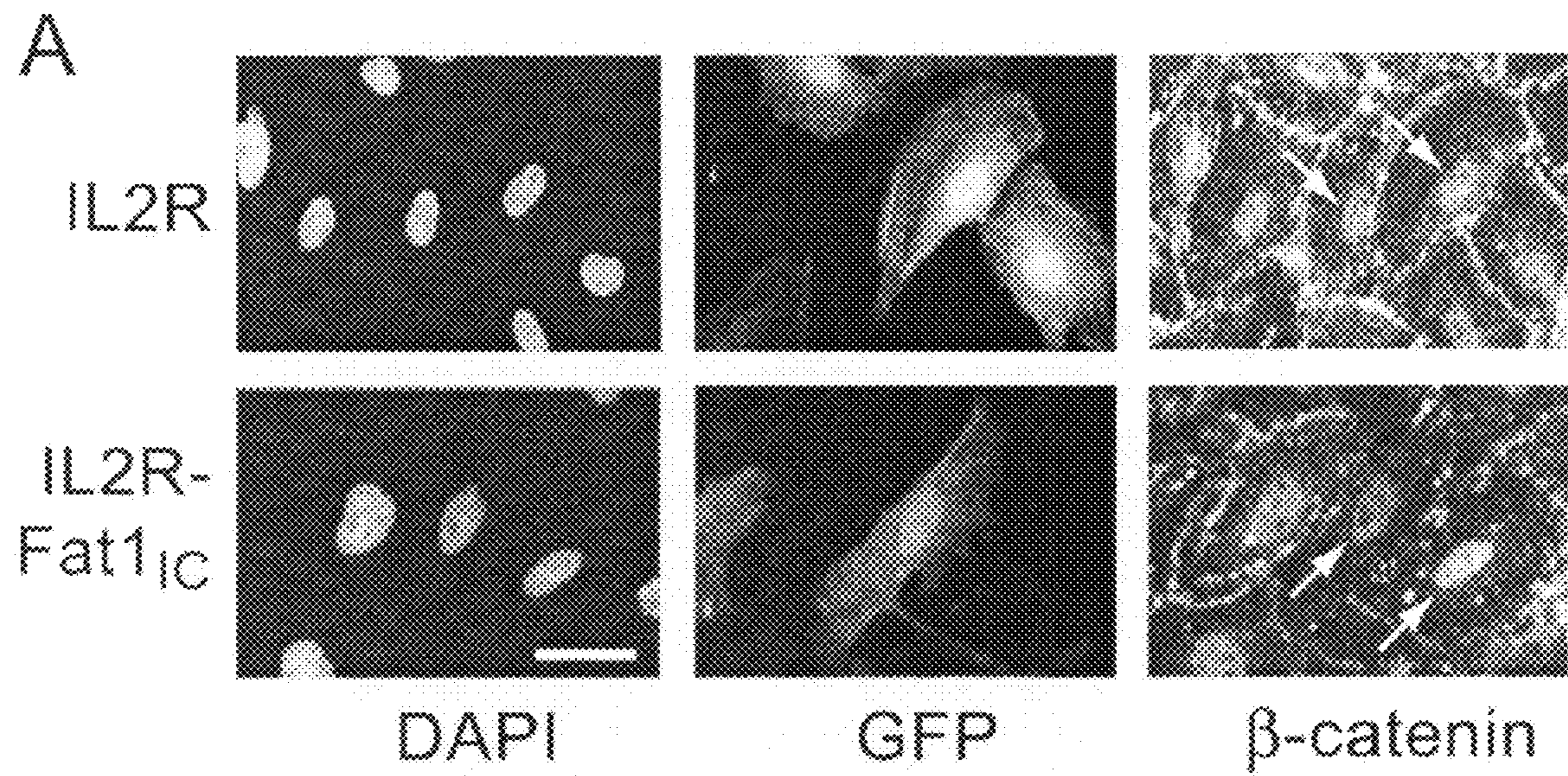


FIG. 9

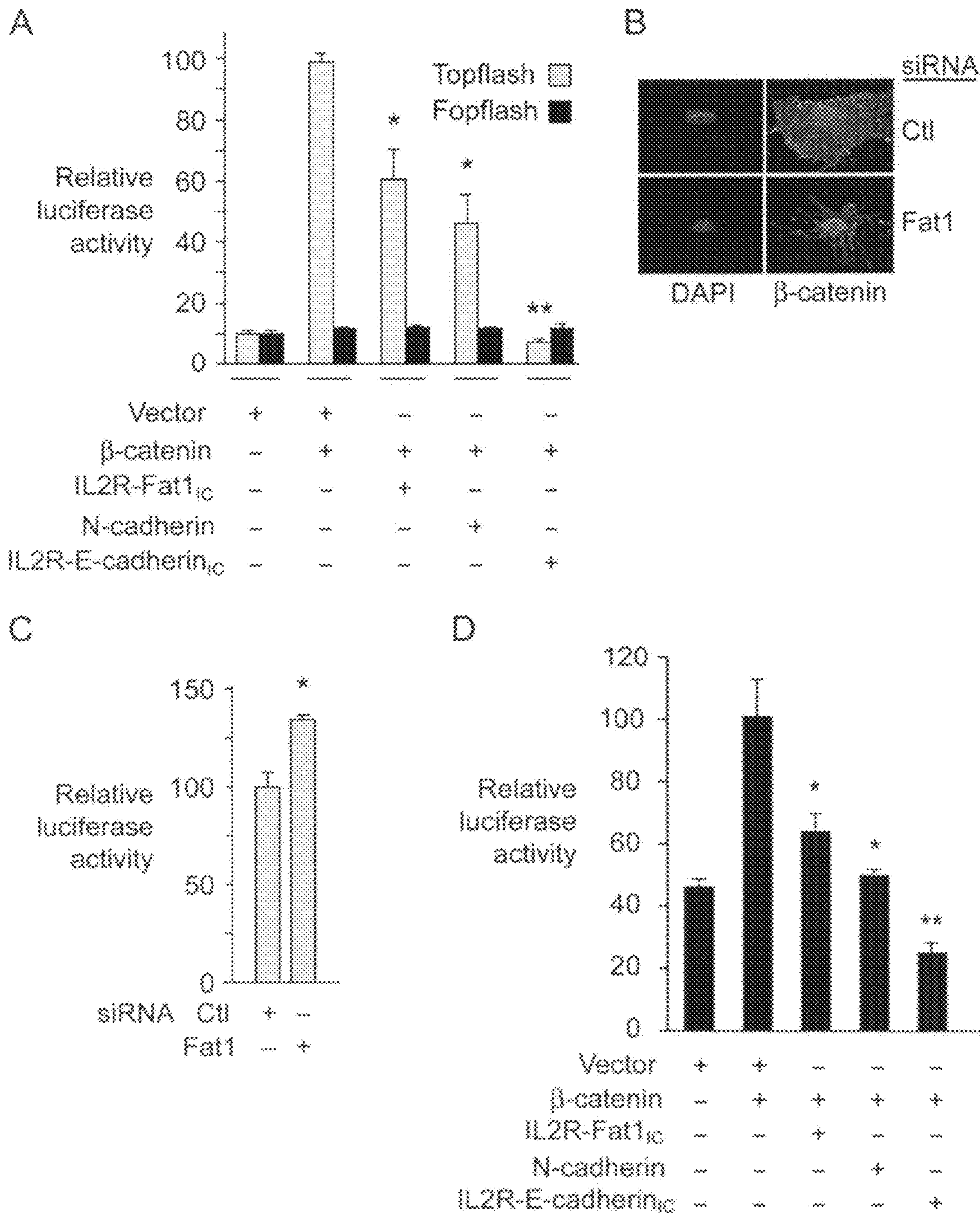
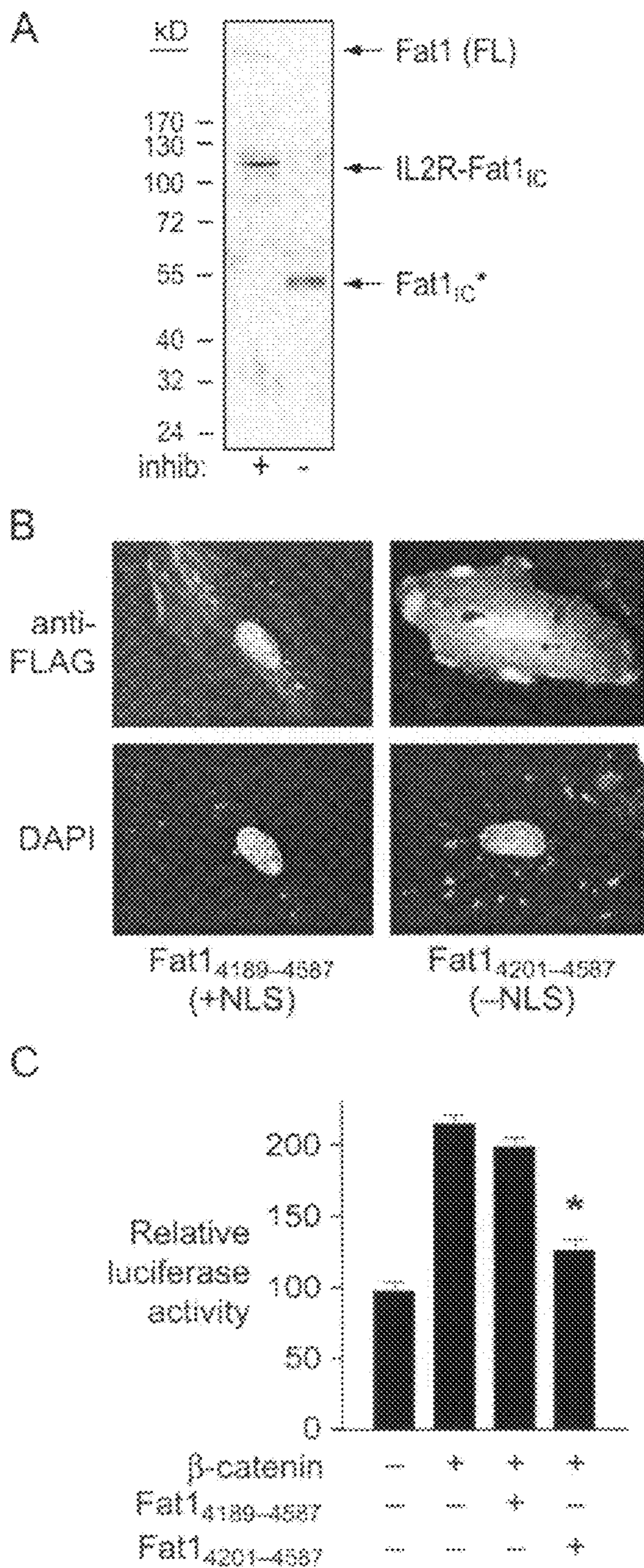


FIG. 10



## 1

INTRACELLULAR DOMAIN OF A  
MAMMALIAN FAT1 (FAT1IC)CROSS REFERENCE TO RELATED  
APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/928,216, filed May 8, 2007, the contents of which are hereby incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY  
SPONSORED RESEARCH OR DEVELOPMENT

The invention was made with government support under grant number R01 HL67944-01 awarded by the National Institutes of Health. The government has certain rights in the invention.

## BACKGROUND OF THE INVENTION

## (1) Field of the Invention

The present invention generally relates to compositions and treatments for vascular disorders. More specifically, the invention provides compositions comprising Fat1 or isolated domains thereof, and methods of using these and other compositions with methods of vascular repair such as angioplasty and coronary bypass surgery.

## (2) Description of the Related Art

Vascular remodeling is a critical part of the pathogenesis of clinically important vascular disorders such as atherosclerosis, restenosis after angioplasty, and saphenous vein graft disease (Shanahan and Weissberg, 1998; Owens et al., 2004). Despite considerable study, the molecular mechanisms that control vascular smooth muscle cell (VSMC) activities during vascular remodeling are not fully understood. Recent reports linking cadherins to VSMC regulation (Jones et al., 2002; Slater et al., 2004; Uglow et al., 2003) suggest that these transmembrane adhesion proteins, characterized extensively as major mediators of epithelial cell homeostasis, may also be important in vascular remodeling.

Cadherins are involved in  $Ca^{2+}$ -dependent cell-cell adhesion, intracellular junction assembly, and tissue morphogenesis during development (Yap et al., 1997; Angst et al., 2001; Wheelock and Johnson, 2003b). Major subdivisions of the large cadherin superfamily include the classical cadherins and the protocadherins (Gallin, 1998; Yagi and Takeichi, 2000; Angst et al., 2001). The extracellular domains of these proteins share a unique structure, the cadherin motif, which is repeated in tandem in variable numbers. Classical cadherins function as homophilic adhesive molecules, and both extracellular and cytoplasmic domains contribute to this function. Classical cadherin cytoplasmic domains interact with  $\beta$ -catenin and plakoglobin (Huber and Weis, 2001; Takeichi, 1995), members of the armadillo gene family of transcription factors. This interaction effectively sequesters  $\beta$ -catenin away from the nucleus, limits its transcriptional activity (Sadot et al., 1998; Kaplan et al., 2001; Simcha et al., 2001), and thus links cadherins to the canonical Wnt signaling pathway, a major determinant of cellular activity during development (Bhanot et al., 1999; Jamora et al., 2003; Nelson and Nusse, 2004).

Like classical cadherins, protocadherins have extracellular domains capable of  $Ca^{2+}$ -dependent, homophilic interaction (Suzuki, 2000). Protocadherin cytoplasmic domains, on the other hand, are structurally divergent from those of the classical cadherins, and less is known about their function.

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Sequestration and inhibition of  $\beta$ -catenin by protocadherins has not been described.

Although mammalian Fat1 genes (Dunne et al., 1995; Ponassi et al., 1999; Cox et al., 2000) were initially characterized as homologues of the *Drosophila* protein Fat (Mahoney et al., 1991), recent bioinformatics analysis indicates that Fat1 is more closely related to *Drosophila* Fat-like (Ftl) (Castillejo-Lopez et al., 2004). In *Drosophila*, Ftl is expressed apically in luminal tissues such as trachea, salivary glands, proventriculus, and hindgut (Castillejo-Lopez et al., 2004). Silencing of ftl results in the collapse of tracheal epithelia, and it has been suggested that Ftl is required for morphogenesis and maintenance of tubular structures of ectodermal origin.

Like *Drosophila* Fat and Ftl, mammalian Fat1 is remarkable for its very large size (~4600 aa). It has a huge extracellular domain that contains 34 cadherin repeats, 5 EGF-like repeats, and 1 laminin A-G motif, a single transmembrane region, and a cytoplasmic tail of ~400 aa (Dunne et al., 1995). Sequences within the Fat1 cytoplasmic domain (Fat1<sub>IC</sub>) show limited similarity to  $\beta$ -catenin binding regions of classical cadherins (Dunne et al., 1995).

## SUMMARY OF THE INVENTION

The inventors have discovered that Fat1 regulates growth and migration of vascular smooth muscle cells (VSMCs).

The invention is directed to an extracellular domain of a mammalian Fat1 (Fat1<sub>EC</sub>), the extracellular domain comprising amino acids equivalent to amino acids 22-4174 of a wild-type mouse Fat1 having the amino acid sequence of SEQ ID NO:1, where the extracellular domain does not comprise the entire mammalian Fat1.

The invention is also directed to an intracellular domain of a mammalian Fat1 (Fat1<sub>IC</sub>), the intracellular domain comprising amino acids equivalent to amino acids 4199-4598 of a wild-type mouse Fat1 having the amino acid sequence of SEQ ID NO:1, where the extracellular domain does not comprise the entire mammalian Fat1.

The invention is additionally directed to a vector comprising a nucleic acid sequence encoding the above-described Fat1<sub>EC</sub>, wherein the vector is capable of expressing the Fat1<sub>EC</sub> in a mammalian cell.

Additionally, the invention is directed to a vector comprising a nucleic acid sequence encoding the above-described Fat1<sub>IC</sub>, wherein the vector is capable of expressing the Fat1<sub>IC</sub> in a mammalian cell.

The invention is further directed to a vascular stent coated with the above-described Fat1<sub>EC</sub>.

Also, the invention is directed to vascular stents coated with a Fat1 ligand that activates Fat1.

Further, the invention is directed to a method of treating a patient at risk for restenosis of a blood vessel, the method comprising inserting the above-described vascular stent into the blood vessel at the site of the risk.

The invention is also directed to a method of treating an injured blood vessel in a patient. The method comprises administering the above-described Fat1<sub>IC</sub> or the above-described Fat1<sub>IC</sub> vector to the injured blood vessel in a manner sufficient to treat the injured blood vessel.

The invention is additionally directed to a method of treating a patient at risk for restenosis of a blood vessel or having an injured blood vessel. The method comprises administering to the patient a vector encoding a Fat1 having an amino acid sequence at least 90% identical to SEQ ID NO:1 or SEQ ID NO:2, where the vector is capable of expressing the Fat1 in

cells of the patient, and wherein the Fat1 is capable of inhibiting growth and promoting migration of vascular smooth muscle cells (VSMC).

The invention is further directed to a method of treating an injured blood vessel in a patient. The method comprises administering a compound to the injured blood vessel, where the compound specifically binds to Fat1 and prevents the ability of the Fat1 to promote migration of vascular smooth muscle cells (VSMC).

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph, a photograph of a western blot, and micrographs of stained tissue sections showing expression of Fat1 in normal and balloon-injured rat carotid arteries. Panel B shows results of a qPCR analysis of fat1 mRNA expression. Fat1 mRNA levels were corrected relative to gapdh mRNA levels, with day 0 (no injury) set=1. \*, P<0.05, \*\*, P<0.01 vs. day 0. Panel B shows the specificity of anti-Fat1 antiserum. Preimmune and anti-Fat1 immune rabbit sera (1:5000 dilutions) were tested by immunoblotting of replicate total RASMC protein extracts (20 µg/lane). The arrow indicates the Fat1 signal. Panel C shows an immunohistochemical analysis of Fat1 expression in arteries 0, 3, 7, and 14 days after injury, as indicated. Arrowheads indicate the internal elastic lamina, and double-ended arrows, the extent of the media. The neointima is the space between arrowhead and arrow. The orientation of all samples is similar, with an asterisk in the day 0 panel indicating the vessel lumen. The upper right panel shows a day 7 sample co-stained for Fat1 and PCNA; areas of increased Fat1 (arrow) and increased PCNA (arrowhead) staining are indicated. The lower right panel shows a day 7 sample in which Fat1 antiserum was omitted (no 1° ab). Scale bar, 100 µm.

FIG. 2 shows photographs of western blots showing a western analysis of Fat1 expression in RASMCs. Cells were serum-deprived, stimulated as described, and harvested for protein extraction at the indicated time points. Actin expression is shown as a loading reference. Data are representative of three independent experiments. Panel A shows induction by 10% FBS for 0-36 h prior to protein extraction. The blot was also probed for cyclin D1. Panel B shows induction by specific factors. Cells were stimulated with ATII, 10<sup>-6</sup> mol/L, bFGF, 20 ng/mL, or PDGF-BB, 20 ng/mL.

FIG. 3 is photographs of western blots, fluorescent and light micrographs of cells, and graphs showing the effect of decreased Fat1 expression on VSMC migration. Panel A shows a western analysis of Fat1 specific and control siRNA efficacy, 48 h after transfection. Panel B shows a schematic diagram of IL2R-Fat1<sub>IC</sub>. EC, extracellular; TM, transmembrane; IC, intracellular, with photomicrograph of subcellular localization of IL2R-Fat1<sub>IC</sub>-3XFLAG in transfected cells stained with FITC-conjugated anti-FLAG antibody. Panel C shows MASMCM migration 30 h after wounding of monolayer. Dashed lines indicate extent of initial denudation. The graphs show quantitative analysis of MASMCM migration after control (Ctl) and specific Fat1 siRNA transfection. For 10 matched fields, the area of the wounded monolayer covered by cells at the indicated timepoints was determined by planimetry using NIH Image, and distance migrated calculated according to the difference from time 0. \*, P<0.05 vs. Ctl siRNA. Panel D shows results of a transwell migration assay of A7r5 cells transduced with the indicated retroviruses. Six fields were counted per condition, and the values were averaged for each filter. \*, P<0.05 vs. other groups.

FIG. 4 is photographs of western blots and a graph showing the effect of decreased Fat1 expression on VSMC cell cycle

progression. Panels A and B show a western analyses of Fat1 and cyclin D1 expression with control (Ctl) or Fat1-specific siRNAs. Panel A shows results when four distinct Fat1-specific siRNAs were transfected 48 h prior to protein extraction. Loading reference, α-tubulin. Panel B shows the efficacy of Ctl or Fat1-specific siRNA 7296 over time. Actin and O-catenin expression were also tested. Panel C shows the effect of Fat1 inhibition on DNA synthesis assessed by BrdU incorporation. Cells were transfected with Ctl or Fat1 siRNA, serum-deprived for 48 h, and stimulated with 10% FBS. BrdU incorporation was assessed as described in Materials and Methods. The graph depicts the means±S.E. of three independent experiments in which a total of 219-874 cells were counted each time for each group. \*, P<0.05 vs. Ctl siRNA.

FIG. 5 is photographs of western blots and graphs showing the effect of IL2R-Fat1<sub>IC</sub> expression on VSMC growth. RV, IL2R, and IL2R-Fat1<sub>IC</sub> designate A7r5 or MASMCMs transduced with the corresponding retroviral constructs. Panel A shows a western analyses of IL2R-Fat1<sub>IC</sub> expression in A7r5 and MASMCM stable transfectants. A non-specific band (ns) near the IL2R-Fat1<sub>IC</sub> protein is indicated. The blots were also probed for cyclin D1 and actin. Panel B shows the effect of IL2R-Fat1<sub>IC</sub> on A7r5 cell growth. Cell number was calculated by CyQuant fluorescence assay by reference to a standard curve. Panel C shows the effect of IL2R-Fat1<sub>IC</sub> on DNA synthesis in A7r5 and MASMCMs, evaluated by BrdU incorporation. \*, P<0.05 vs. control.

FIG. 6 is fluorescence micrographs and photographs of western blots showing the co-localization and interaction of endogenous β-catenin and Fat1 in VSMCs. Panel A shows an immunofluorescence analysis of β-catenin (β-cat), Fat1, and areas of co-localization (“merge”). Nuclei were stained with DAPI as indicated. β-catenin and Fat1 co-localization at cell-cell junctions is indicated by an arrowhead. Scale bar (10 µm) applies to all panels. Panel B shows detail from the panels in A, showing staining for Fat1 (right micrograph), but not β-catenin (left), at the cellular free edge. Scale bar, 10 µm. Panel C shows the co-immunoprecipitation of endogenous β-catenin and Fat1. Cell lysates were incubated with antibodies specific for Fat1 (upper panels) or β-catenin (lower panels) or normal rabbit or mouse IgG, and the immunoprecipitated complexes were analyzed by western blot for Fat1 and β-catenin, as indicated.

FIG. 7 is a diagram and photographs of western blots showing the identification of β-catenin-interacting residues in the Fat1<sub>IC</sub> domain by co-immunoprecipitation of epitope-tagged proteins. Panel A is a schematic depiction of FLAG-tagged IL2R-Fat1<sub>IC</sub> deletion constructs. Fat1<sub>IC</sub> domains indicated: juxtamembrane (JM), FC1, FC2, carboxy-terminus (CT). Panel B shows a western analysis of immunoprecipitated protein complexes. The indicated constructs were transfected into 293T cells. Upper blots: after 24 h, total cellular protein (400 µg) was harvested and analysed by immunoprecipitation and western blotting (WB) with antibodies against the epitope tags, as indicated. Lower blots: protein input (7.5%). An analogous E-cadherin-derived construct (IL2R-E-cad<sub>IC</sub>-FLAG) was used as a positive control.

FIG. 8 is fluorescence micrographs and photographs of western blots showing the effect of Fat1<sub>IC</sub> overexpression on β-catenin nuclear localization in VSMCs. Panel A shows immunofluorescence analysis of β-catenin subcellular localization in IL2R-GFP-RV (upper) and IL2R-Fat1<sub>IC</sub>-GFP-RV (lower) transduced RASMCs. Cells were treated with LiCl (20 mmol/L) for 6 h, and then stained with anti-β-catenin antibody and DAPI. Transduced cells were identified by co-expressed GFP. Arrows indicate nuclear β-catenin signal of untransduced and transduced cells within each panel (see

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text). Scale bar, 10  $\mu$ m. Panel B shows western analysis of  $\beta$ -catenin in membrane (M), cytoplasmic (C), and nuclear (N) fractions extracted from IL2R-GFP-RV and IL2R-Fat1<sub>IC</sub>-GFP-RV transduced A7r5 cells treated with LiCl, as above. The blot was probed for lamin A/C to assess fractionation and loading.

FIG. 9 is graphs and fluorescent micrographs showing the effect of altered Fat1 expression on  $\beta$ -catenin transcriptional activity in VSMCs. Panel A shows Topflash (TCF-luciferase reporter) activation. Topflash or Fopflash control was transfected into A7r5 cells along with expression constructs for  $\beta$ -catenin, IL2R-Fat1<sub>IC</sub>, N-cadherin, and/or IL2R-E-cadherin<sub>IC</sub>. The maximal reporter activity was set to 100. \*, P<0.05, \*\*, P<0.01, vs. activity with  $\beta$ -catenin alone. Panel B shows  $\beta$ -catenin localization in MAMSCs transfected with control (Ctl, scrambled) or Fat1-specific (7296) siRNAs and stimulated with LiCl (20 mM) for 12 h. Panel C shows Topflash activity with decreased Fat1 expression. MAMSCs transfected with the indicated siRNAs and the Topflash reporter were stimulated with LiCl (20 mM) for 12 h prior to assay for luciferase activity. \*, P<0.05, vs. activity with Ctl siRNA. Panel D shows Cyclin D1 promoter activation. The cyclin D1 promoter-luciferase construct was transfected into A7r5 cells along with test constructs, as in A. \*, P<0.05, \*\*, P<0.01, vs. activity with  $\beta$ -catenin alone.

FIG. 10 is a photograph of a western blot, fluorescent micrographs, and a graph showing cleavage, localization, and activity of Fat1 cytoplasmic sequences in VSMC protein extracts. Panel A shows western analysis of A7r5 extracts transduced with IL2R-Fat1<sub>IC</sub> retrovirus. Total cellular protein was incubated at 37°C. for 15 min with or without proteinase inhibitors (inhib). Both full length (FL) Fat1 and the fusion protein (IL2R-Fat1<sub>IC</sub>), are apparent with proteinase inhibition; only a single band (Fat1<sub>IC</sub>\*) of ~50 kD is seen without inhibition. Panel B shows subcellular localization of the FLAG-tagged Fat1 cytoplasmic domain with (Fat1<sub>4189-4587</sub>) or without (Fat1<sub>4201-4587</sub>) the putative NLS in transfected A7r5 cells. Anti-FLAG immunofluorescence and DAPI nuclear stains are shown. Panel C shows the effect of the NLS on Fat1<sub>IC</sub>-mediated inhibition of  $\beta$ -catenin activation of the cyclin D1 promoter. Luciferase activity was assessed 24 h after transfection of A7r5 cells with the indicated expression constructs and the cyclin D1 promoter reporter. \*, P<0.01 vs. activity with  $\beta$ -catenin alone.

## DETAILED DESCRIPTION OF THE INVENTION

The inventors have discovered that Fat1 regulates growth and migration of vascular smooth muscle cells (VSMCs). See Example. In particular, it was discovered that the complete Fat1 protein inhibits proliferation (i.e., growth) and promotes migration of VSMCs and the intracellular domain (i.e., cytoplasmic fragment) of Fat1 inhibits both proliferation and migration of VSMCs. These discoveries make useful various invention compositions and enable various therapeutic methods.

The invention is directed to an extracellular domain of a mammalian Fat1 (Fat1<sub>EC</sub>), the extracellular domain comprising amino acids equivalent to amino acids 22-4174 of a wild-type mouse Fat1 having the amino acid sequence of SEQ ID NO:1, where the extracellular domain does not comprise the entire mammalian Fat1.

As used herein, a Fat1 is FAT tumor suppressor homolog 1 (Drosophila) having UniProtKB/TrEMBL entry Q9QXA3. The mouse Fat1 wild-type amino acid sequence is provided herein as SEQ ID NO:1 and the mouse wild-type Fat1 mRNA sequence is provided in Genbank accession no. NM\_00\_

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001081286. The human wild-type Fat1 amino acid sequence is provided herein as SEQ ID NO:2 and the human wild-type Fat1 in RNA sequence is provided in Genbank accession no. NM\_005245.3. It is expected that any other wild-type mammalian Fat1 amino acid sequence could be determined by the skilled artisan. Such an amino acid sequence would be expected to be at least 85% identical to SEQ ID NO:1 and/or SEQ ID NO:2.

The Fat1 protein is made up of a signal peptide consisting of residues 1-21 of both SEQ ID NO:1 and SEQ ID NO:2; an extracellular domain consisting of residues 22-4174 of SEQ ID NO:1 and 22-4181 of SEQ ID NO:2; a transmembrane domain consisting of residues 4175-4198 of SEQ ID NO:1 and 4182-4201 of SEQ ID NO:2; and an intracellular domain consisting of residues 4199-4598 of SEQ ID NO:1 and 4202-4588 of SEQ ID NO:2.

The Fat1 or fragments thereof claimed herein or used in the methods claimed herein includes mutants comprising amino acid deletions, substitutions or additions, provided the mutant inhibits proliferation (i.e., growth) and promotes migration of VSMCs and the intracellular domain (i.e., cytoplasmic fragment) of the mutant inhibits both proliferation and migration of VSMCs. Methods for determining these characteristics are provided, e.g., in the Example.

The invention Fat1<sub>EC</sub> can further comprise other amino acids or other compounds. Examples of additional amino acids includes the signal sequence, or a portion of the transmembrane domain. Examples of another compound that can usefully be bound to the invention Fat1<sub>EC</sub> is a fluorescent dye.

The invention Fat1<sub>EC</sub> is preferably a mouse Fat1 having an amino acid sequence at least 95% identical to SEQ ID NO:1. More preferably, the invention Fat1<sub>EC</sub> is a human Fat1 having an amino acid sequence at least 95% identical to SEQ ID NO:2. Most preferably, the invention Fat1<sub>EC</sub> is capable of inhibiting growth and promoting migration of vascular smooth muscle cells (VSMC).

When used for therapeutic purposes, the invention Fat1<sub>EC</sub> is preferably in a pharmaceutically acceptable carrier.

By "pharmaceutically acceptable" it is meant a material that (i) is compatible with the other ingredients of the composition without rendering the composition unsuitable for its intended purpose, and (ii) is suitable for use with subjects as provided herein without undue adverse side effects (such as toxicity, irritation, and allergic response). Side effects are "undue" when their risk outweighs the benefit provided by the composition. Non-limiting examples of pharmaceutically acceptable carriers include, without limitation, any of the standard pharmaceutical carriers such as phosphate buffered saline solutions, water, emulsions such as oil/water emulsions, microemulsions, and the like.

The above-described mutant Fat1<sub>EC</sub> can be formulated without undue experimentation for administration to a mammal, including humans, as appropriate for the particular application. Additionally, proper dosages of the compositions can be determined without undue experimentation using standard dose-response protocols.

Although the Fat1<sub>EC</sub> can be easily formulated for oral, lingual, sublingual, buccal, intrabuccal, rectal, or nasal administration, it is preferred that they be formulated for parenteral administration, such as for example, by intravenous, intramuscular, intrathecal or subcutaneous injection, since that is the most preferred route of administration of these proteins. Parenteral administration can be accomplished by incorporating the compounds into a solution or suspension. Such solutions or suspensions may also include sterile diluents such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol

or other synthetic solvents. Parenteral formulations may also include antibacterial agents such as for example, benzyl alcohol or methyl parabens, antioxidants such as for example, ascorbic acid or sodium bisulfite and chelating agents such as EDTA. Buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose may also be added. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

The invention is also directed to an intracellular domain of a mammalian Fat1 (Fat1<sub>IC</sub>), the intracellular domain comprising amino acids equivalent to amino acids 4199-4598 of a wild-type mouse Fat1 having the amino acid sequence of SEQ ID NO:1, where the extracellular domain does not comprise the entire mammalian Fat1.

Preferably, the invention Fat1<sub>IC</sub> is a mouse Fat1 having an amino acid sequence at least 95% identical to SEQ ID NO:1. More preferably, the Fat1 is a human Fat1 having an amino acid sequence at least 95% identical to SEQ ID NO:2. Most preferably, the Fat1<sub>IC</sub> is capable of inhibiting both growth and migration of vascular smooth muscle cells (VSMC).

The Fat1<sub>IC</sub> here can further usefully be fused to an amino acid sequence to make a fusion protein capable of localizing the Fat1<sub>IC</sub> to a cell membrane. See Example. Preferably, the fusion amino acid sequence is a transmembrane region of an interleukin 2 receptor  $\alpha$ -chain.

When used for therapeutic purposes, the invention Fat1<sub>EC</sub> is preferably in a pharmaceutically acceptable carrier, as described above.

The above-described invention Fat1<sub>EC</sub> and Fat1<sub>IC</sub> can also be provided as a vector for transfection of mammalian cells. Thus, the invention is additionally directed to a vector comprising a nucleic acid sequence encoding the above-described Fat1<sub>EC</sub>, where the vector is capable of expressing the Fat1<sub>EC</sub> in a mammalian cell.

Also, the invention is directed to a vector comprising a nucleic acid sequence encoding the above-described Fat1<sub>IC</sub>, wherein the vector is capable of expressing the Fat1<sub>IC</sub> in a mammalian cell.

The above Fat1<sub>EC</sub> can be applied to a vascular stent. Such a stent is useful for therapeutic applications (further discussed below). The ability to inhibit growth of VSMCs but not endothelial cells is useful for insertion into a blood vessel or vein where there is a risk for restenosis, since having an intact endothelium reduces the risk for thrombosis when compared to the common drug-eluting stents, which also inhibit endothelial cells. Thus, the invention is further directed to a vascular stent coated with the above-described Fat1<sub>EC</sub> that is capable of inhibiting growth and promoting migration of VSMC.

The benefits of the Fat<sub>EC</sub>-coated stents discussed immediately above can also be achieved by instead coating the stent with a Fat1 ligand that activates Fat1. Thus, the invention is also directed to vascular stents coated with a Fat1 ligand that activates Fat1. The Fat1-activating ligand can be identified by, e.g., screening a library of compounds for the ability to activate Fat1. Preferably the library comprises antibodies (e.g., monoclonal antibodies or phage display antibodies). A preferred Fat1 ligand is an antibody, e.g., identified by that screening procedure.

The above compositions are particularly useful in methods of treating patients that have undergone angioplasty, bypass surgery or other similar procedures. In one aspect of therapeutic methods, the invention is directed to a method of treating a patient at risk for restenosis of a blood vessel, the method comprising inserting the above-described vascular

stent coated with the Fat1<sub>EC</sub> into the blood vessel at the site of the risk. Preferably here, the blood vessel is an artery, most preferably a coronary artery.

The invention is also directed to a method of treating an injured blood vessel in a patient. The method comprises administering the above-described Fat1<sub>IC</sub> or the above-described Fat1<sub>IC</sub> vector to the injured blood vessel in a manner sufficient to treat the injured blood vessel. Where the vector is used, it is administered such that it can transfect VSMC cells in the blood vessel. In these methods, the administered Fat1<sub>IC</sub>, or the Fat1<sub>IC</sub> synthesized from the vector, is useful in preventing restenosis due to the ability of the Fat1<sub>IC</sub> to inhibit VSMC proliferation. Optionally, the Fat1<sub>IC</sub> can further comprise the above-described amino acid sequence that targets the Fat1<sub>IC</sub> to the cell membrane. Preferably, the blood vessel is an artery or vein used in a coronary artery bypass surgery.

The invention is additionally directed to a method of treating a patient at risk for restenosis of a blood vessel or having an injured blood vessel. The method comprises administering to the patient a vector encoding a Fat1 having an amino acid sequence at least 90% identical to SEQ ID NO:1 or SEQ ID NO:2, where the vector is capable of expressing the Fat1 in cells of the patient, and wherein the Fat1 is capable of inhibiting growth and promoting migration of vascular smooth muscle cells (VSMC). The Fat1 expressed from the vector would inhibit growth and facilitate migration of VSMCs. Preferably here the Fat1 is a human Fat1 having an amino acid sequence at least 95% identical to SEQ ID NO:2. More preferably, the Fat1 is a human Fat1 having an amino acid sequence at least 99% identical to SEQ ID NO:2. The vector is also preferably administered to the patient during or after coronary artery bypass surgery. Alternatively, the vector is administered to the patient during or after angioplasty.

The invention is further directed to a method of treating an injured blood vessel in a patient. The method comprises administering a compound to the injured blood vessel, where the compound specifically binds to Fat1 and prevents the ability of the Fat1 to promote migration of vascular smooth muscle cells (VSMC). Such an application is useful to limit migration and oppose VSMC accumulation in arterial neointima.

In some aspects of these methods, the compound comprises an antibody binding site. Preferably, the compound is an antibody that has previously been shown to inhibit Fat1 activity. Such antibodies can be prepared without undue experimentation. Alternatively, the compound is an aptamer. These methods are most preferably used where the blood vessel is an artery or vein used in a coronary artery bypass surgery.

Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims, which follow the examples.

## Example

The Fat1 cadherin integrates vascular smooth muscle cell growth and migration signals.

## Example Summary

This Example is based on the publication Hou et al., 2006.

The significance of cadherin superfamily proteins in vascular smooth muscle cell (VSMC) biology is undefined. Described here are recent studies of the Fat1 protocadherin. Fat1 expression in VSMCs increases significantly after arterial injury or growth factor stimulation. Fat1 knockdown decreases VSMC migration in vitro, but surprisingly, enhances cyclin D1 expression and proliferation. Despite limited similarity to classical cadherins, the Fat1 cytoplasmic domain (Fat1<sub>IC</sub>) interacts with  $\beta$ -catenin, inhibiting both its nuclear localization and transcriptional activity. Fat1 undergoes cleavage and Fat1<sub>IC</sub> species localize to the nucleus; however, inhibition of the cyclin D1 promoter by truncated Fat1<sub>IC</sub> proteins corresponds to their presence outside the nucleus, which argues against repression of  $\beta$ -catenin-dependent transcription by nuclear Fat1<sub>IC</sub>. These findings extend recent observations about Fat1 and migration in other cell types, and demonstrate for the first time its anti-proliferative activity and interaction with O-catenin. Because it is induced after arterial injury, Fat1 may control VSMC functions central to vascular remodeling by facilitating migration and limiting proliferation.

## Introduction

These studies show that Fat1 expression increases after injury of the rat carotid artery, and is positively regulated in cultured VSMCs by several factors that promote cell proliferation and migration. Interestingly, knockdown of Fat1 expression limits VSMC migration, but enhances VSMC growth. This anti-proliferative effect of Fat1 appears to be mediated by Fat1<sub>IC</sub> sequences, since expression of a fusion protein containing the Fat1<sub>IC</sub> inhibits cyclin D1 expression and cell growth. Moreover, the Fat1<sub>IC</sub> can interact with O-catenin, prevent its nuclear translocation, and limit its transcriptional activity on both synthetic and native  $\beta$ -catenin-responsive promoters, including that of cyclin D1, a known target of canonical Wnt signaling. These findings point to an integrative role for Fat1 in regulation of critical VSMC activities, in which it promotes migration and limits both canonical Wnt signaling and VSMC growth in the remodeling artery.

## Results

## Expression of Fat1 Increases after Arterial Injury.

fat1 mRNA expression was quantified by quantitative PCR (qPCR) of cDNA samples from normal and injured rat carotid arteries. Compared to uninjured arteries, fat1 mRNA expression was ~8.5-, 13.0-, and 3.9-fold higher than control at 3, 7, and 14 days after injury, respectively (FIG. 1A).

To localize Fat1 protein expression in injured arteries, we characterized rabbit antisera raised against a GST-Fat1<sub>IC</sub> immunogen. Immunoblotting of VSMC lysates with one such antiserum, but not preimmune serum, yielded a single high molecular weight band of ~500 kD, in accord with the predicted size of full length Fat1 (FIG. 1B). Further specificity was demonstrated in RNA interference experiments directed against multiple separate targets in the mouse Fat1 sequence (FIGS. 3 and 4). The antiserum was then used for immunohistochemical studies. As shown in FIG. 1C, prominent Fat1 staining appeared in the media 3 days after injury, while at 7 days and 14 days after injury, Fat1 staining was less evident in the media, but clearly present in the developing neointima.

Western analysis of Fat1 expression in the carotid artery injury model, like our qPCR findings, showed a clear induction after injury (data not shown). To correlate Fat1 expression with the proliferative status of specific cells, we co-stained sections for Fat1 and the proliferation marker PCNA. While some cells appeared positive for both, we also noted some spatial separation of the signals, particularly evident in areas with limited neointimal formation, which showed prominent Fat1 staining without PCNA (FIG. 1C, upper right). The latter observation raised the possibility that, despite its overall induction after injury, increased Fat1 expression might have negative effects on VSMC growth in vivo (FIG. 1C, upper right).

## Serum and Growth Factors Induce Fat1 Expression in VSMCs.

To identify factors that might contribute to Fat1 induction after arterial injury, its expression was characterized in primary cultured VSMCs. Quiescent rat aortic smooth muscle cells (RASMCs) (time 0 h) were treated with 10% FBS for 2, 6, 12, 18, 24, and 36 h, and the level of Fat1 protein was determined by Western analysis. The Fat1 signal increased strongly between 2 and 12 h and remained elevated through 36 h (FIG. 2A). To assess cell cycle status, we also checked cyclin D1 expression in these lysates. Interestingly, Fat1 induction preceded the increase of cyclin D1, a mediator of progression through the G1 phase of the cell cycle (FIG. 2A).

We then assessed Fat1 expression in response to several factors known to affect the vascular response to injury. Western analyses showed that expression of Fat1 increased in response to Angiotensin II (ATII), basic FGF (bFGF), and PDGF-BB (FIG. 2B). Increased Fat1 expression was apparent by 2 h and sustained at high levels from 12 to 36 h after stimulation with each of these factors. Thus, Fat1 expression is regulated consistently and strongly by multiple factors known to promote VSMC growth and migration.

## Inhibition of Fat1 Expression Limits VSMC Migration.

Two recent studies have described a role for Fat1 in regulation of epithelial cytoskeletal actin dynamics, planar polarity, and migration, mediated through interactions of the Fat1 cytoplasmic domain with proteins of the Ena/VASP family (Moeller et al., 2004; Tanoue and Takeichi, 2004). Fat1 induction by known VSMC chemotactic factors (FIG. 2) suggested that Fat1 might also be involved in VSMC migration. To test this and other potential Fat1 functions, we developed reagents to effectively manipulate Fat1 expression. Transfection of mouse aortic smooth muscle cells (MASMCs) with Fat1 specific small interfering RNAs (siRNAs), but not scrambled or mismatch derivatives, resulted in significantly decreased levels of Fat1 protein (FIG. 3A). To isolate and augment signals mediated by the Fat1<sub>IC</sub>, we generated a cDNA construct, IL2R-Fat1<sub>IC</sub>, in which the entire Fat1 cytoplasmic domain was fused to the extracellular domain and transmembrane region of the interleukin 2 receptor x-chain (IL2R), with or without a C-terminal FLAG epitope tag (FIG. 3B). Subcellular localization of this fusion protein was tested in 3T3 cells, which do not express detectable Fat1, and A7r5 VSMCs, which express moderate amounts of endogenous Fat1; both transfected 3T3 and A7r5 cells showed an appropriate cell surface signal when stained with anti-FLAG epitope antibody (FIG. 3B and data not shown). Cell migration in monolayers treated with specific Fat1 siRNA was modestly but significantly decreased compared with control siRNA (FIG. 3C), which indicates that Fat1 expression is required for optimal VSMC migration. Surprisingly, we also found decreased migration of VSMCs expressing the IL2R-Fat1<sub>IC</sub> protein in a Transwell assay using FBS as a stimulant in the lower chamber (FIG. 3D). Both expression of Ena/VASP proteins in



VSMCs, and the ability of the IL2R-Fat1<sub>IC</sub> protein to interact with these signaling intermediates was confirmed (data not shown). It is surmised that although the IL2R-Fat1<sub>IC</sub> construct may increase intracellular Fat1 signaling, it also disassociates Fat1 extracellular interactions from this intracellular signaling, and thus interferes with directional migration. Altogether, these findings indicate that Fat1 promotes VSMC migration; it is likely that, as described in epithelial cells, interactions with Ena/VASP proteins link Fat1 expression to VSMC cytoskeletal actin reorganization, polarization, and migration.

#### Inhibition of Fat1 Expression Promotes VSMC Growth.

In addition to increased migration, the VSMC response to injury is characterized by cell cycle entry and increased proliferation (Clowes et al., 1983a). To evaluate how Fat1 induction after injury might affect VSMC growth, the effect of Fat1 knockdown on expression of cyclin D1 (a marker of cell cycle activation) was tested. Four distinct mouse Fat1 siRNA duplexes attenuated endogenous Fat1 levels in MASMCS; with each duplex, a significant increase in cyclin D1 expression over control levels was also observed (FIG. 4A and data not shown). The similarity of effect achieved by multiple distinct siRNAs argues strongly that increased cyclin D1 expression results from decreased Fat1, and not an off-target effect. The duration of Fat1 inhibition was more than 90% at 2 and 3 days after transfection, with persistent and strong inhibition still apparent after 6 days (FIG. 4B). Decreased Fat1 expression corresponded to increased cyclin D1 signal at each time point (2.0-2.5-fold increase of cyclin D1/actin ratio vs. control), suggesting that endogenous Fat1 exerts a tonic inhibitory effect on cyclin D1 expression (FIG. 4B). The level of total  $\beta$ -catenin in these cells, by contrast, showed little change.

The effect of Fat1 knockdown on DNA synthesis was also examined. Cells were transfected with Fat1 or control siRNA, and then serum deprived for 48 h prior to stimulation with 10% FBS and evaluation of BrdU incorporation. In Fat1 knockdown cultures, the fraction of BrdU positive cells was significantly higher than in control siRNA cells (52 $\pm$ 7% vs. 30 $\pm$ 8%, P<0.05) (FIG. 4C). These findings indicate that decreased Fat1 expression promotes cell cycle progression and DNA synthesis in VSMCs.

#### The Fat1<sub>IC</sub> is Sufficient to Inhibit VSMC Growth.

Classical cadherins interact with intracellular signaling pathways through their cytoplasmic domains (Wheelock and Johnson, 2003a). To establish cell populations differing primarily in their expression of the Fat1<sub>IC</sub>, the IL2R (without cytoplasmic domain) and IL2R-Fat1<sub>IC</sub> constructs were transferred into the GFP-RV retroviral vector (Ranganath et al., 1998). Viral supernatants were produced and used to transduce A7r5 and primary MASMCS. Additional control cells, denoted RV, were produced using the unmodified GFP-RV vector. Western analysis confirmed IL2R-Fat1<sub>IC</sub> expression in A7r5 and MASMCS (FIG. 5A). Interestingly, endogenous cyclin D1 levels were lower in both A7r5 and MASMCS expressing IL2R-Fat1<sub>IC</sub> (FIG. 5A). In cell growth assays over 7 days, A7r5 cells expressing IL2R showed no significant change from control RV cells, but decreased cell numbers were evident in the IL2R-Fat1<sub>IC</sub> at all timepoints after 3 days (FIG. 5B). In addition, both A7r5 and MASMCS expressing the IL2R-Fat1<sub>IC</sub> construct showed significantly lower fractions of BrdU-positive nuclei, indicating that this decrease in cell number reflected growth inhibition rather than decreased survival (FIG. 5C). Fat1 and  $\beta$ -Catenin co-localize and interact in VSMCs. In epithelial cells, classical cadherins such as E-cadherin regulate Wnt signaling activity by physically associating with  $\beta$ -catenin at points of cell-cell contact

(Nathke et al., 1994). The sequences, interacting proteins, and functions of protocadherin cytoplasmic domains are typically thought to be divergent from those of the classical cadherins (Yagi and Takeichi, 2000), and Fat1 is not regarded as part of the classical cadherin system (Tanoue and Takeichi, 2005). Nevertheless, we found that the Fat1<sub>IC</sub> has growth inhibitory activity, and that expression of cyclin D1, a known target of the canonical Wnt signaling pathway, correlated negatively with Fat1<sub>IC</sub> expression. Together these findings suggested that growth inhibition by Fat1 might involve  $\beta$ -catenin. In our immunofluorescent analyses of RASMCs (FIG. 6), Fat1 localized to both cell-cell junctions and cellular free edges, while  $\beta$ -catenin was concentrated at sites of cell-cell contact. By two color immunofluorescence analysis, we found areas along cell-cell junctions where the two signals overlapped (FIG. 6A). This overlap did not include the cellular free edges, where Fat1 alone was seen (FIG. 6B).

Junctional  $\beta$ -catenin and Fat1 have been identified in epithelial cells that display apical-basal polarity, but it is thought that the two proteins occupy distinct domains, with  $\beta$ -catenin at apical adherens junctions and Fat1 at basolateral points of cell-cell contact (Tanoue and Takeichi, 2004; Tanoue and Takeichi, 2005). VSMCs are non-polarized (Muller and Gimbrone, 1986), so this model of apical-basal domain specialization may not apply. To test directly if Fat1 and  $\beta$ -catenin can interact at physiologic levels of expression in VSMCs, endogenous Fat1 was immunoprecipitated. Recovery of  $\beta$ -catenin was also determined. Both this assay and reciprocal co-immunoprecipitations of  $\beta$ -catenin followed by immunoblotting for Fat1 demonstrated interaction of the two proteins (FIG. 6C). This finding suggests that the non-polarized nature of VSMCs allows for protein-protein interactions not found in polarized cell types such as epithelial cells. Further immunoblotting of Fat1 immunoprecipitates with a pan-cadherin antibody did not reveal associated (classical) cadherins that might associate with both Fat1 and  $\beta$ -catenin (data not shown).

To characterize the Fat1- $\beta$ -catenin interaction further, co-immunoprecipitation assays were used in co-transfected 293T cells to map the sequences required for interaction. A series of constructs bearing deletions within the Fat1<sub>IC</sub> portion of the IL2R-Fat1<sub>IC</sub>-3XFLAG were generated (FIG. 7A). IL2R-E-cadherin<sub>IC</sub>-3XFLAG and IL2R-3XFLAG (containing no Fat1 sequences) constructs served as positive and negative controls, respectively. The expression of Myc-tagged  $\beta$ -catenin and FLAG-tagged fusion proteins was confirmed, as was immunoprecipitation of transfected Myc-tagged  $\beta$ -catenin (FIG. 7B, lower panels). Interaction of  $\beta$ -catenin with the IL2R-Fat1<sub>IC</sub>-3XFLAG derivatives was assessed by immunoblotting with FLAG antibody (FIG. 7B, upper panel). A robust FLAG signal was obtained with the IL2R-Fat1<sub>IC</sub>-3XFLAG construct containing the complete Fat1<sub>IC</sub> domain and with derivatives I, III, and V. Weaker signals were seen with constructs II and IV, which lack the FC1 and both FC1 and FC2 domains, respectively. While these findings based on overexpressed proteins must be interpreted with caution, they suggest that  $\beta$ -catenin interacts with the Fat1<sub>IC</sub> principally through the FC1 domain, but leave open the possibility that the FC2 domain or additional sequences also contribute to the interaction. Interestingly, the E-cadherin-based positive control yielded a comparatively strong band, despite input of substantially less protein.

#### Expression of the Fat1<sub>IC</sub> Affects $\beta$ -Catenin Cellular Distribution and Transcriptional Activity.

As noted above, changes in Fat1 or Fat1<sub>IC</sub> expression affected expression of a  $\beta$ -catenin target gene, cyclin D1, but had little effect on overall  $\beta$ -catenin levels (FIG. 4B). Having

found evidence for co-localization and interaction of  $\beta$ -catenin and Fat1 in VSMCs, it was postulated that Fat1 might be acting like a classical cadherin to affect the subcellular localization and activity of  $\beta$ -catenin. This was first examined using immunocytochemistry. Expression plasmids encoding IL2R or IL2R-Fat1<sub>IC</sub> were introduced into VSMCs, which were subsequently treated with LiCl (20 mM) for 6 h to activate Wnt signaling and promote nuclear translocation of  $\beta$ -catenin (Hedgepeth et al., 1997). The intensity of nuclear  $\beta$ -catenin staining did not appear to be affected by expression of IL2R (arrows, FIG. 8A, upper panel). In contrast, nuclear accumulation of  $\beta$ -catenin appeared decreased in the IL2R-Fat1<sub>IC</sub>-expressing cells (arrows, FIG. 8A, lower panel), as compared with untransfected cells. To assess this effect in a more quantitative way, the distribution of  $\beta$ -catenin was determined in the membrane, cytoplasmic, and nuclear fractions of IL2R-GFP-RV and IL2R-Fat1<sub>IC</sub>-GFP-RV transduced VSMC cultures treated with LiCl. As shown in FIG. 8B, immunoblotting showed a relative decrease in nuclear  $\beta$ -catenin accumulation in cells expressing IL2R-Fat1<sub>IC</sub>, as compared with those expressing IL2R (respective nuclear  $\beta$ -catenin/lamin A/C ratios 0.8 (IL2R-Fat1<sub>IC</sub>) vs 1.65 (IL2R)).

To assess further the functional significance of the Fat1- $\beta$ -catenin interaction in VSMCs, we tested the effect of Fat1<sub>IC</sub> overexpression on  $\beta$ -catenin-mediated transcription. A7r5 cells were co-transfected with  $\beta$ -catenin and/or IL2R-Fat1<sub>IC</sub>, along with the TCF-luciferase reporter construct Topflash or its negative control, Fopflash (FIG. 9A). Topflash reporter activity reflects activation of the canonical Wnt signaling pathway,  $\beta$ -catenin nuclear translocation, and formation of TCF/ $\beta$ -catenin heterodimers; Fopflash contains mutated TCF binding sites and serves as a control for non-specific activation (Korinek et al., 1997). A full-length N-cadherin cDNA and the IL2R-E-cadherin<sub>IC</sub> construct were also tested as controls. Specific activation of Topflash by  $\beta$ -catenin was ~10-fold above basal levels, and the three test constructs all inhibited this activation significantly. Interestingly, the inhibition due to both IL2R-Fat1<sub>IC</sub> (40%) and N-cadherin (55%) was less complete than that resulting from co-transfection of IL2R-E-cadherin<sub>IC</sub>, which abolished all  $\beta$ -catenin-mediated transactivation. We also evaluated the effect of decreased Fat1 expression. Immunocytochemistry of LiCl-stimulated MAMSCs suggested a relative enhancement of nuclear  $\beta$ -catenin staining in Fat1-depleted cells (FIG. 9B). To assess this observation more quantitatively, we transfected MAMSCs first with control or Fat1-specific siRNA and then with the Topflash reporter. As shown in FIG. 9C, LiCl-stimulated TCF/ $\beta$ -catenin transcriptional activation was ~30% higher in Fat1 knockdown cells compared with control.

As shown in FIGS. 4 and 5, cyclin D1 levels varied inversely with the level of Fat1<sub>IC</sub> expression. The cyclin D1 promoter is a known transcriptional target of Wnt signaling and activated TCF/ $\beta$ -catenin complexes (Shutman et al., 1999; Tetsu and McCormick, 1999), so we postulated that Fat1<sub>IC</sub> might also inhibit the native cyclin D1 promoter. VSMCs were co-transfected with  $\beta$ -catenin and/or IL2R-Fat1<sub>IC</sub>, along with the cyclin D1 promoter-luciferase reporter construct (Herber et al., 1994). N-cadherin and the IL2R-E-cadherin<sub>IC</sub> fusion protein were also tested. Most of the  $\beta$ -catenin-mediated activation of the cyclin D1 promoter reporter was eliminated by IL2R-Fat1<sub>IC</sub> or N-cadherin expression (FIG. 9D). Consistent with the Topflash results, IL2R-E-cadherin<sub>IC</sub> was more effective, as it decreased promoter activity to a level below baseline.

Inhibition of  $\beta$ -Catenin Activity Depends on Extracellular Localization of the Fat1<sub>IC</sub>.

Fat1 is a type I transmembrane protein, and immunofluorescence studies with antiserum specific for Fat1<sub>IC</sub> sequences showed expression at the cell surface, as expected (FIG. 6). We also noted consistent signals in the cell nucleus with this antiserum. This observation, together with a recent report of localization of Fat1 cytoplasmic sequences to the nucleus (Magg et al., 2005), raised the possibility that inhibition of  $\beta$ -catenin by Fat1 might result from a nuclear (transcriptional repressor) function of a cleaved Fat1<sub>IC</sub> fragment, rather than sequestration of  $\beta$ -catenin outside the nucleus. Indeed, incubation without proteinase inhibitors of extracts of A7r5 cells expressing both native Fat1 and the IL2R-Fat1<sub>IC</sub> fusion protein showed the disappearance of these full length proteins and rapid appearance of a single, relatively stable species of ~50 kD (FIG. 10A). Because the N-terminus of this cleaved product is not yet defined, we designate it as Fat1<sub>IC</sub>\*; its apparent size in SDS-PAGE suggests that it contains most, if not all, of the ~400 aa Fat1<sub>IC</sub> domain.

Like human Fat1<sub>IC</sub> (Magg et al., 2005), the mouse Fat1<sub>IC</sub> contains a potential nuclear localizing sequence (NLS) (RK-MISRKKKR) near its N-terminus. The effect of this sequence on Fat1<sub>IC</sub> localization was tested by immunocytochemical analysis of A7r5 cells transfected with FLAG-tagged expression constructs that retain (Fat1<sub>4189-4587</sub>) or exclude (Fat1<sub>4201-4587</sub>) the NLS motif. Fat1<sub>4189-4587</sub> localized almost exclusively to the nucleus, while Fat1<sub>4201-4587</sub> was apparent in the nucleus and prominent throughout the cytoplasm (FIG. 10B).

To evaluate these findings in the context of Fat1-mediated VSMC growth inhibition, these Fat1<sub>IC</sub> derivatives were tested for effects on cyclin D1 promoter activity. The IL2R-Fat1<sub>IC</sub> fusion protein yielded significant inhibition of  $\beta$ -catenin-mediated cyclin D1 promoter activation (FIG. 9D, above); Fat1<sub>4201-4587</sub>, but not Fat1<sub>4189-4587</sub>, retained this inhibitory effect (FIG. 10C). Both Fat1<sub>4201-4587</sub> and Fat1<sub>4189-4587</sub> are present in the nucleus, but the former has a cytoplasmic distribution not shared by Fat1<sub>4189-4587</sub>; hence, we attribute this inhibitory effect on  $\beta$ -catenin to the extracellular presence of Fat1<sub>4201-4587</sub>.

#### Discussion

Fat1 is expressed widely during mouse and rat development (Cox et al., 2000; Ponassi et al., 1999), notably in areas with high levels of cellular proliferation. Although in situ hybridization of rat embryos demonstrated expression of fat1 mRNA in the developing aortic outflow tract (Ponassi et al., 1999), the significance of Fat1 in vascular tissues has not been explored previously.

We found relatively low expression of Fat1 in normal adult rat carotid arteries, and substantially increased levels during the first few days after injury (FIG. 1A). Immunohistochemical analyses (FIG. 1C) showed prominent Fat1 staining first in the injured arterial media, and subsequently in the neointima, a pattern of expression similar to that of VSMC proliferation in this model (Clowes et al., 1983b). Interestingly, areas of attenuated neointimal formation showed prominent Fat1 and decreased PCNA staining, providing an initial suggestion that Fat1 might act to limit VSMC proliferation in vivo (FIG. 1C). Nevertheless, Fat1 levels in cultured VSMCs increased in response to serum and several factors known to promote VSMC activation and neointimal formation, including ATII (Powell et al., 1990), PDGF-BB (Ferns et al., 1991), and bFGF (Lindner and Reidy, 1991) (FIG. 2). This expression pattern contrasts with that described for N-cadherin, which decreases after stimulation of VSMC with serum or PDGF-

BB (Ugnow et al., 2003), and that of R-cadherin, which decreases substantially in the first few days after injury (Slater et al., 2004).

To evaluate how induction of this very large protocadherin might affect the response to vascular injury, we tested the effect of Fat1 on VSMC migration and proliferation, two of the key cellular functions activated in this setting. Both loss of Fat1 expression and expression of the IL2R-Fat1<sub>IC</sub> fusion protein attenuated VSMC migration (FIG. 3). In the context of recent reports regarding Fat1 function in epithelial cells (Moeller et al., 2004; Tanoue and Takeichi, 2004), these findings suggest that increased Fat1 expression facilitates VSMC migration by providing directional cues and stimulating actin cytoskeletal remodeling through its interactions with proteins of the Ena/VASP family. Together with the Fat1 knockdown results, inhibition of migration by the IL2R-Fat1<sub>IC</sub> fusion protein suggests that dissociation of Fat1 extracellular interactions from Fat1<sub>IC</sub>-mediated intracellular signaling interferes with directional migration.

Despite the induction of Fat1 in the proliferative phase after injury and in response to growth factor stimulation of cultured cells, our results in both loss- and gain-of-function studies (FIGS. 4, 5) suggest that Fat1 opposes VSMC proliferation. Loss of growth suppression resulting in imaginal disc overgrowth in *Drosophila* led to identification of Fat (Mahoney et al., 1991), the founding member of the cadherin subfamily that includes mammalian Fat1. While recent analyses indicates that mammalian Fat1 is more closely related to *Drosophila* Ftl (Castillejo-Lopez et al., 2004) than to Fat, a growth regulatory function has yet to be described for Ftl. Altered growth characteristics were also not identified in mouse Fat1<sup>-/-</sup> neural progenitors and embryonic skin (Ciani et al., 2003). Thus, our findings in VSMCs may reflect cell type-specific differences in the expression of cadherins or other protocadherins functionally redundant with Fat1, or differences in the level of  $\beta$ -catenin expression. In either case, the results of Fat1 knockdown studies indicate that in VSMCs, endogenous levels of Fat1 expression are sufficient to limit cyclin D1 expression (FIG. 4) and  $\beta$ -catenin-mediated transcription (FIG. 9), while our gain-of-function studies (FIG. 5) suggest that decreased cyclin D1 expression and cell growth are likely physiologic consequences of Fat1 induction. Cyclin D1, a known TCF/ $\beta$ -catenin target gene (Shtutman et al., 1999; Tetsu and McCormick, 1999), plays a critical role in regulation of G1 phase progression and G1/S cell cycle transition (Jiang et al., 1993; Resnitzky et al., 1994), and the level of its expression is closely controlled. Increased Fat1 expression in response to injury probably acts to slow VSMC proliferation, at least in part by decreasing cyclin D1 expression.

Signaling by classical cadherins has been studied extensively, but the mechanisms of protocadherin signaling are not well understood. The intracellular portion of Fat1 shows limited similarity to classical cadherin cytoplasmic domains, with 30 of 137 (22%) residues matching consensus in the FC1 domain and 28 of 84 (33%) residues matching consensus in the FC2 domain (Dunne et al., 1995). Although Tanoue and Takeichi described partial co-localization of Fat1 and  $\beta$ -catenin in immortalized epithelial cell lines, they found more O-catenin in apical lateral cell contacts and more Fat1 in basal lateral cell contacts (Tanoue and Takeichi, 2004), and concluded that Fat1 does not participate in the classical cadherin system (Tanoue and Takeichi, 2005). Interestingly, these findings are consistent with the observation that in polarized epithelial cells, complexes forming between adjacent cells vary in composition according to their apical vs. basal position (Johnston and Gallant, 2002). Thus our findings in

VSMCs, which are morphologically and biochemically non-polarized (Muller and Gimbrone, 1986), may differ because of the lack of apical-basal specialization in this cell type. In immunocytochemical studies, we found that  $\beta$ -catenin and Fat1 co-localized in a junctional pattern at points of contact between VSMCs (FIG. 5); Fat1 staining was also observed at cellular free edges, while O-catenin was not.

It is believed that a physical interaction between endogenous Fat1 and O-catenin has not been demonstrated previously. Clear evidence was found that these proteins interact at physiologic levels of expression. Transfection studies with the IL2R-Fat1<sub>IC</sub> fusion protein indicated that, despite limited similarity to the  $\beta$ -catenin-interacting domains of classical cadherins, the Fat1<sub>IC</sub> domain was sufficient for this interaction (FIG. 7). While mapping studies suggested that the Fat1 FC1 domain was most important for the  $\beta$ -catenin-Fat1 interaction, deletion of other domains within the Fat1<sub>IC</sub> also decreased the amount of protein co-immunoprecipitation, indicating that sequences both within and outside of the relatively conserved FC1 and FC2 domains may contribute to  $\beta$ -catenin-Fat1 interaction. Interestingly, the FC1 domain corresponds to the area of greatest similarity (54/196 aa identity, (27%)) with the *Drosophila* Ftl cytoplasmic domain; its role in the O-catenin-Fat1 interaction described here suggests that Ftl may be capable of interaction with armadillo, the *Drosophila* homologue of  $\beta$ -catenin.

The IL2R-Fat1<sub>IC</sub> chimera allowed functional analyses without confounding effects attributable to increased expression of the Fat1 extracellular domain. Expression of IL2R-Fat1<sub>IC</sub>, but not a control protein lacking the Fat1<sub>IC</sub> domain, decreased nuclear translocation of O-catenin (FIG. 8), and inhibited  $\beta$ -catenin transactivation of both synthetic (Topflash) and native (cyclin D1) TCF-dependent promoters (FIG. 9). Although we found evidence of Fat1 cleavage resulting in a Fat1<sub>IC</sub>\* fragment that may localize to the nucleus (FIG. 10), only a defined Fat1<sub>IC</sub> fragment lacking the NLS (aa 4189-4198) reproduced the inhibitory effect of the IL2R-Fat1<sub>IC</sub> fusion protein. This result suggests that inhibition of  $\beta$ -catenin transcriptional activity is mediated by Fat1<sub>IC</sub> outside the nucleus, and is not due to Fat1<sub>IC</sub> peptides in the nucleus. Thus, it remains to be determined if cleavage and nuclear translocation of Fat1<sub>IC</sub> underlies a specific function, perhaps as a chaperone or transcriptional regulator, or if it is important as a means to inactivate Fat1-mediated inhibition of  $\beta$ -catenin. Our studies to date indicate that the interaction of Fat1 cytoplasmic sequences with  $\beta$ -catenin has consequences for overall regulation of VSMC growth. The underlying mechanism appears similar to that described for classical cadherin-mediated sequestration of  $\beta$ -catenin in epithelial cells (Orsulic et al., 1999), but in the case of the protocadherin Fat1, this mechanism may be operative only in non-polarized cells such as VSMCs.

These findings suggest that increased expression of Fat1 after vascular injury facilitates migration and opposes proliferation of VSMCs. The former effect likely involves Fat1 interaction with Ena/VASP proteins, as described in other cell types (Moeller et al., 2004; Tanoue and Takeichi, 2004), while the latter effect relies on decreased nuclear accumulation of  $\beta$ -catenin (this study). Interestingly, Fat1<sub>IC</sub> interaction with, and inhibition of O-catenin both appeared less robust than that observed with classical cadherin sequences (FIGS. 7, 9), suggesting that Fat1 may be less efficient than the classical cadherins at sequestering  $\beta$ -catenin. Fat1 induction after injury and by growth factors contrasts with the expression pattern of other cadherins found in VSMCs. Together, these observations suggest that Fat1 may guide VSMC migration while remaining relatively permissive of growth in settings when VSMC proliferation is necessary for vascular repair.

*Drosophila* Ftl is thought to use its exceptionally large extracellular domain to promote epithelial cell separation during formation of tubular organs in embryogenesis (Castillejo-Lopez et al., 2004); it may be that mammalian Fat1, by virtue of its similar structure, may expedite circumferential distribution of VSMCs around the injured artery. Altogether, it is tempting to speculate that Fat1 limits VSMC proliferation while providing directional migration cues important during vascular remodeling, providing an integrative function that may oppose the formation of hyperproliferative cellular clusters. Finally, though expression of Fat1 in human vascular disease has not yet been evaluated, it is possible that loss of Fat1-mediated negative regulation could contribute to VSMC hyperplastic syndromes such as restenosis, transplant arteriopathy, or vein graft disease.

#### Materials and Methods

##### Rat Carotid Artery Balloon Injury.

All procedures were in accordance with institutional guidelines. The rat carotid artery balloon injury model was implemented as described (Sibinga et al., 1997). Briefly, male Sprague-Dawley rats (20 in total, Zivic-Miller) weighing 350 to 400 g were anesthetized with ketamine (40 mg/kg) and xylazine (5 mg/kg). The left common carotid artery was denuded of endothelium and stretched by three passages of a 2F embolectomy catheter according to standard protocols. At 3, 7 and 14 days after injury, animals were reanesthetized and killed, and carotid arteries were harvested and snap-frozen in liquid nitrogen for RNA and protein extraction, or fixed with 4% PFA and processed for paraffin embedding for immunohistochemical analysis.

##### qPCR.

A cDNA fragment identified in differential mRNA display analysis of the rat carotid artery injury model (Sibinga et al., 1997) was cloned, sequenced, and subjected to BLAST analysis, which revealed homology of the sequence fragment with the 3' end of the rat Fat1 ORF (Genbank NM\_031819). Total RNA was extracted from vascular tissues by homogenization in TRIzol (Invitrogen), treated with DNase 1 (1 U/ $\mu$ l, Promega), and used for first-strand cDNA synthesis. The mRNA levels were quantified in triplicate by qPCR in the Mx3000P Real-Time PCR System with the Brilliant SYBR Green qPCR kit (Stratagene). Rat Fat1 specific primers for qPCR were 5'-CCCCTTCCAACCTCTCCCTCA-3' (forward) (SEQ ID NO:3) and 5'-CAGGCTCTCCCGGGCACTGT-3' (reverse) (SEQ ID NO:4). PCR cycling conditions included 10 min at 95° C. for 1 cycle followed by 45 cycles at 95° C. for 30 s, 60° C. for 30 s, and 72° C. for 60 s. Dissociation curve analysis confirmed that signals corresponded to unique amplicons. Expression levels were normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in RNA levels for each sample, obtained from parallel assays and analyzed using the comparative  $\Delta\Delta C_t$  method (Bustin, 2000).

##### Western Analysis.

Fat1-specific antisera were raised in rabbits. A cDNA fragment encoding mouse Fat1 aa 4434-4587 was generated by PCR and cloned in frame with GST in the pGEX-2T plasmid. The resultant fusion protein was expressed in bacteria, purified by GST-sepharose affinity chromatography (Pharmacia), and used as an immunogen in a standard rabbit injection protocol (Cocalico Labs). Fat1-specific antiserum was purified by affinity chromatography performed sequentially on a GST column and a GST-Fat1 column. Antiserum specificity was evaluated by Western analysis of GST-Fat1 fusion protein and whole cell lysates from RASMCs (1:5000 dilution).

Other mouse antibodies used were anti- $\beta$ -catenin (1:100, E-5, Santa Cruz), anti-cyclin D1 (1:100, DCS-6, NeoMarkers), anti-FLAG M2 (1:5000, Sigma), and anti-c-myc (1:250, 9E10).

For protein analyses, cells or vascular tissue samples were homogenized and extracted in RIPA buffer with or without protease inhibitors. Whole cell lysate (30  $\mu$ g) was separated by electrophoresis through 3-8% Novex Tris-acetate or 4-12% Bis-Tris polyacrylamide gels (Invitrogen) and transferred to Immobilon-P membrane (Millipore). After blocking in TBST (Tris pH 8.0, NaCl 150 mmol/L, and 0.1% Tween-20) plus 4% (w/v) non-fat milk, blots were incubated overnight at 4° C. with primary antibodies. The blots were then incubated with HRP-conjugated secondary antibody and activity was visualized by enhanced chemiluminescence (ECL, Amersham). Equivalent protein loading was evaluated with anti- $\alpha$ -tubulin (1:500, NeoMarkers), anti-lamin A/C (1:100, N-18, Santa Cruz) or anti-actin (1:100, C-11, Santa Cruz) antibodies.

##### Immunohistochemistry.

Rat carotid arterial sections (5  $\mu$ m) were incubated overnight with anti-Fat1 antiserum (1:2000), washed extensively, and incubated with a 1:500 dilution of secondary antibody (biotinylated goat anti-rabbit IgG, DAKO). Slides were incubated with avidin and biotinylated HRP, developed with a peroxidase substrate solution (DAKO), and counterstained with hematoxylin (Fisher). Specificity of staining was confirmed by omission of the primary antibody. PCNA staining was performed with anti-PCNA (1:100, PC 10, LabVision), alkaline phosphatase-conjugated goat anti-mouse secondary antibody (1:200), and visualization with BM Purple substrate (Roche). Images were obtained using an Eclipse E600 microscope, 40 $\times$ /NA 0.75 Plan objective, and Coolpix 5400 camera (Nikon).

##### Cell Culture.

Primary culture RASMCs were prepared as described (Sibinga et al., 1997) and maintained in Dulbecco's MEM (DMEM, Invitrogen) containing 10% FBS (HyClone), 100 U/mL penicillin, 100  $\mu$ g/mL streptomycin, and 10 mmol/L HEPES (pH 7.4, Sigma). RASMCs were passaged every 3 to 5 days, and used between 4 and 8 passages from harvest. Primary culture MASMCs were harvested from the aortas of 12 week old male FVB mice by enzymatic dissociation, evaluated by immunocytochemical analysis by using  $\alpha$  smooth muscle actin antibody (1:400, Clone 1A4, NeoMarkers) and maintained in DMEM containing 10% FBS, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin. MASMCs were passaged every 2 to 4 days, and used between 4 and 8 passages from harvest. The A7r5 embryonic RASMC, 3T3, and 293T cell lines (American Tissue Type Collection) were cultured in DMEM containing 10% FBS. ATII was obtained from Sigma, and bFGF and PDGF-BB from Collaborative Biomedical. In stimulation experiments, the cells were made quiescent by incubation in medium containing 0.4% horse serum for 72 h prior to addition of the FBS or growth factor. Control cultures received an equivalent amount of vehicle. Whole cellular protein was extracted at designed time points.

##### RNA Interference.

The mouse Fat1 short interfering RNA (siRNA) templates were comprised of 19 bp sense sequences derived from GenBank Accession AJ250768 (position 4881, 5'-GGACCGAAGTCACCAAGTA-3' [SEQ ID NO:5]; position 5126, 5'-GCGACGCATTTAACATTAA-3' [SEQ ID NO:6]; position 6432, 5'-GCATGACACTTTAAATAAA-3' [SEQ ID NO:7]; position 7296; 5'-GTCTGGCAATGATCATAAA-3') [SEQ ID NO:8] followed by a 9 bp loop sequence, a 19 bp antisense sequence, and a T7 promoter sequence. Control

siRNAs included scrambled (GTAACCATAAACAG-GCATT—SEQ ID NO:9) and mismatched (GTCTGATAATGCGCATAAAA—SEQ ID NO:10) derivatives of the 7296 sequence, and an unrelated siRNA based on the Renilla luciferase sequence. siRNA was transcribed in vitro using the T7-MEGAscript™ kit (Ambion), and transfected with X-tremeGENE Reagent (Roche) according to manufacturer recommendations. Fat1 knockdown efficiency was assessed by Western analysis.

#### cDNA Constructs.

The mouse Fat1<sub>IC</sub> cDNA was generated by RT-PCR with primers containing HindIII and XbaI sites (underlined) to facilitate cloning: forward 5'-AAGCTTCTCTGCCGGAAGATGATCAGTCGG-3' (SEQ ID NO:11) and reverse 5'-TCTAGACACTTCCGTATGCTGCTGGGA-3' (SEQ ID NO:12). The product was subcloned into the p3XFLAG-CMV-14 expression vector (Sigma). The IL2R expression construct (LaFlamme et al., 1994) was used to construct a chimeric cDNA encoding the IL2R extracellular and transmembrane domains and the Fat1<sub>IC</sub>, with or without an in frame 3XFLAG tag (IL2R-Fat1<sub>IC</sub>-3XFLAG and IL2R-Fat1<sub>IC</sub>, respectively). The IL2R-E-cadherin<sub>IC</sub>-3XFLAG construct was produced using a similar strategy. The truncated FLAG-tagged Fat1<sub>IC</sub> constructs, Fat<sub>4189-4587</sub> and Fat<sub>14201-4587</sub>, were generated by PCR from the IL2R-Fat1<sub>IC</sub>-3XFLAG template using forward primers 5'-CCATGGGCCTCTGCCGGAAGATGATCAGT-3' (SEQ ID NO:13) and 5'-CCATGGGCCAGGCTGAACCTGAAGACAAAC-3' (SEQ ID NO:14) and the CMV24 reverse primer; the resulting fragments were cloned into pcDNA3.1v5 (Invitrogen). The FLAG-tagged N-cadherin and Myc-tagged β-catenin constructs were gifts from R. Hazan and R. Kemler, respectively. All constructs were confirmed by sequencing.

#### Retrovirus Preparation and Transduction.

The retrovirus system used is based on the IRES-GFP-RV constructs developed by K. Murphy (Washington University, St. Louis) and Phoenix ecotropic packing cells provided by G. Nolan (Stanford University). The IL2R-Fat1<sub>IC</sub> cDNA was inserted upstream of the encephalomyocarditis virus internal ribosomal entry sequence (IRES) and green fluorescent protein (GFP) ORF in the GFP-RV vector. A7r5 cells, MASMCS, or RASMCs ( $5 \times 10^5$ ) were infected with virus-containing supernatant in the presence of polybrene (8 μg/mL). Control cells transduced with virus encoding GFP alone or IL2R and GFP were generated in parallel, and FACS analysis of retroviral transduced cell lines indicated similar levels of GFP expression.

#### Cell Migration Assays.

Cell migration was assessed by 1) scratch wounding of monolayers and 2) with Transwell 24-well cell culture inserts with 8-μm pores (Costar). For the former, MASMCS transfected with control or Fat1-specific siRNA were grown to confluence, and monolayers were denuded similarly using a 1000 μl pipette tip. Photomicrographs of the same fields were obtained sequentially at 24 and 30 h after injury using a Nikon TMS microscope, Plan 4×/NA 0.13 DL objective, and Coolpix 5400 camera, and cellular progress was quantitated by planimetry of the denuded area and converted to distance migrated using NIH Image 1.63 software. For Transwell assays, quiescent cells were harvested, counted, and added ( $5 \times 10^4$ /well) to the insert. Culture medium containing 10% FBS as chemotactic agent was added to the lower chamber. After 4 h, non-migrating cells were removed from upper filter surfaces, and the filter was washed, fixed, and stained. Six

randomly selected 200× fields were then photographed and cells that had migrated to the underside of the filter were counted.

#### Cell Proliferation Assays.

Cell number was evaluated with the CyQUANT Assay (Molecular Probes). Cells ( $2 \times 10^4$  per well) were plated in 6-well plates in DMEM containing 2% FBS, medium was replaced every other day, and at each time point, triplicate wells were washed with PBS and frozen at  $-80^\circ$  C. Net sample fluorescence was determined on a Victor 2 plate reader (Wallac) and enumerated by reference to a standard curve. For the bromodeoxyuridine (BrdU) incorporation assay, cells plated on chamber slides (Becton-Dickinson) were serum-starved (0.4% horse serum) for 48 h and then stimulated with 10% FBS. BrdU (10 μM, Sigma) was added to cells for 6 h prior to harvest at 24 h. Cells were washed in PBS, fixed in 4% PFA, treated with HCl, and stained sequentially with anti-BrdU antibody (1:200, Abcam) and Alexa Fluor 555 conjugated secondary antibody (1:2000, Molecular Probes). Cells were counterstained with DAPI (Molecular Probes). Signals were visualized by fluorescence microscopy, and the numbers of BrdU-positive and total nuclei per field calculated.

#### Immunocytochemistry.

Cells were plated on chamber slides 24 h prior to staining, and then washed with PBS, fixed with PFA, blocked with 3% normal goat serum, and incubated with anti-β-catenin (1:100) and anti-Fat1 (1:1000) antibodies. Specific staining was identified with goat anti-mouse and chicken anti-rabbit IgG (Alexa Fluors, Molecular Probes). Expression of FLAG-tagged proteins was detected using FITC-conjugated anti-FLAG M2 antibody (8 μg/ml, Sigma). After counterstaining with DAPI, samples were mounted (Supermount medium, Biogenex) on glass slides and signals were visualized using an Olympus IX70 inverted fluorescent microscope equipped with 20×/NA 0.4 and 40×/NA 0.6 LWD objectives and standard fluorescent filter sets, a Cooke Sencam CCD camera, and IPLab software (Scanalytics). Subsequent image processing was performed using Photoshop 7.0 and Illustrator 10.0 (Adobe Systems). Routine control experiments included omission of the primary antibodies. For Wnt pathway activation, cells were treated with LiCl (20 mmol/L) for 6-12 h, and then stained with anti-β-catenin antibody and DAPI nuclear stain.

#### Co-Immunoprecipitation.

Deletions within the Fat1<sub>IC</sub> portion of the IL2R-Fat1<sub>IC</sub>-3XFLAG construct were engineered using the vector XbaI site and introducing NheI restriction sites (Quikchange mutagenesis, Stratagene) in frame at the following positions in the mouse Fat1 aa sequence: 4187, 4244, 4395, and 4497. The sequences between selected pairs of restriction sites were excised, plasmids recircularized, and constructs confirmed by sequencing. Plasmids were introduced into 293T cells using Lipofectamine 2000 (Invitrogen). Whole cell lysates were harvested 24 h after transfection in lysis buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 0.1% sodium deoxycholate, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM NaF, with protease inhibitors. Myc-tagged β-catenin was immunoprecipitated by incubating 400 μg of precleared lysate with 2 μg of c-Myc antibody for 2 h at 4° C., followed by incubation with protein G agarose (Invitrogen) at 4° C. overnight. For immunoprecipitation of endogenous proteins, RASMC whole cell lysates were precleared and then incubated with anti-Fat1 antiserum, anti-β-catenin antibody, or normal rabbit or mouse IgG for 2 h at 4° C., followed by incubation with protein G agarose overnight. The beads were washed and immune complexes recovered by boiling in sample buffer.

Fat1 and  $\beta$ -catenin were detected by Western analysis, as described above.

#### Cell Fractionation.

Membrane, cytoplasmic, and nuclear fractions were prepared using the Compartment Protein Extraction Kit (Chemicon) according to the manufacturer's instructions. Fractionation and loading of proteins was evaluated by western analysis with anti-lamin A/C antibody (Santa Cruz).

#### Analysis of Reporter Gene Activation.

A7r5 cells growing in DMEM supplemented with 10% FBS were transfected transiently using Lipofectamine 2000 with  $\beta$ -catenin, IL2R-Fat1<sub>IC</sub>, Fat<sub>4189-4587</sub>, Fat1<sub>4201-4587</sub> or control expression constructs, along with the TCF wild type (Topflash) and mutated control (Fopflash) luciferase reporter plasmids (Upstate Biotechnology), or cyclin D1 promoter luciferase reporter (Herber et al., 1994). MASMCS were transfected by Amaxa electroporation according to the manufacturer's instructions. The total amount of transfected DNA

was kept constant. Cell lysates were harvested 24 h after transfection, and luciferase activity was determined using the Glo-lysis buffer system (Promega) and the Victor 2 plate reader. Luciferase activities were normalized to protein levels for each well. The data shown represent transfections repeated at least three times each.

#### Statistical Analysis.

Experiments were repeated at least three times. Data are presented as mean $\pm$ SEM. Comparisons between 2 groups were analyzed by Student's t test, and comparisons between 3 or more groups were assessed by analysis of variance (ANOVA) with a Bonferroni/Dunn post hoc test. Significance was accepted for values of P<0.05.

#### Abbreviations List:

IC, intracellular; MASMCS, mouse aortic smooth muscle cell; qPCR, quantitative PCR; RASMC, rat aortic smooth muscle cell; siRNA, small interfering RNA; VSMC, vascular smooth muscle cell.

#### SEQ ID NOS

SEQ ID NO: 1. Mouse Fat1 amino acid sequence - deduced from Refseq  
Genbank accession NM\_001081286 (see also Genbank NP\_001074755.1)

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SEQ ID NO:2. Human Fat1 amino acid sequence - deduced from Refseq

Genbank accession NM\_005245.3 (see also Genbank NP\_005236.2)

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SEQ ID NO:4. Rat Fat1-specific reverse primer  
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SEQ ID NO:5. Mouse Fat1 siRNA template  
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SEQ ID NO:6. Mouse Fat1 siRNA template  
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SEQ ID NO:7. Mouse Fat1 siRNA template  
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SEQ ID NO:8. Mouse Fat1 siRNA template  
 GTCTGGCAATGATCATAAA

SEQ ID NO:9. Control siRNA  
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SEQ ID NO:10. Control siRNA  
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SEQ ID NO:11. Mouse Fat1<sub>IC</sub> RT-PCR forward primer. Restriction site  
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SEQ ID NO:12. Mouse Fat1<sub>IC</sub> RT-PCR reverse primer. Restriction site  
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SEQ ID NO:13. Fat<sub>4189-4587</sub> PCR forward primer. Restriction site  
 is underlined.  
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SEQ ID NO:14. Fat<sub>14201-4587</sub> PCR forward primer  
CCATGGGCCAGGCTGAACCTGAAGACAAAC. Restriction site is underlined.

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In view of the above, it will be seen that the several advantages of the invention are achieved and other advantages attained.

As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

All references cited in this specification are hereby incorporated by reference. The discussion of the references herein is intended merely to summarize the assertions made by the authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.

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                           645                          650                          655

Pro Leu Tyr Ile Asn Leu Thr Val Ala Ala Ser Arg Lys Pro Val Asn  
                           660                          665                          670

Leu Gln Cys Glu Glu Thr Gly Val Ala Lys Met Leu Ala Glu Lys Leu  
       675                          680                          685

Leu Gln Ala Asn Lys Leu His Ser Gln Gly Asp Val Glu Asp Ile Phe

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690			695			700									
Phe	Asp	Ser	Tyr	Ser	Val	Asn	Thr	His	Thr	Pro	Gln	Phe	Gly	Val	Thr
705					710					715					720
Leu	Pro	Thr	Gly	Ile	Glu	Val	Lys	Glu	Asn	Leu	Pro	Val	Gly	Ala	Asn
				725					730					735	
Ile	Leu	Phe	Met	Asn	Ala	Thr	Asp	Leu	Asp	Ser	Gly	Phe	Asn	Gly	Lys
			740					745					750		
Leu	Val	Tyr	Ala	Ile	Ser	Gly	Gly	Asn	Asp	Asp	Ser	Cys	Phe	Thr	Ile
		755					760					765			
Asp	Met	Glu	Thr	Gly	Val	Leu	Lys	Val	Leu	Ser	Pro	Leu	Asp	Arg	Glu
	770						775				780				
Val	Met	Asp	Lys	Tyr	Thr	Leu	Asn	Ile	Thr	Val	Tyr	Asp	Leu	Gly	Ile
785					790					795					800
Pro	Gln	Arg	Ala	Ala	Trp	Arg	Leu	Leu	Asp	Val	Thr	Val	Leu	Asp	Ala
			805						810					815	
Asn	Asp	Asn	Ala	Pro	Glu	Phe	Leu	Gln	Glu	Ser	Tyr	Phe	Val	Glu	Val
			820					825					830		
Ser	Glu	Asp	Lys	Glu	Val	Asn	Ser	Glu	Ile	Ile	Gln	Val	Glu	Ala	Thr
		835					840					845			
Asp	Lys	Asp	Leu	Gly	Pro	Ser	Gly	His	Val	Thr	Tyr	Ala	Ile	Leu	Thr
	850						855				860				
Asp	Thr	Glu	Lys	Phe	Ser	Ile	Asp	Ser	Met	Thr	Gly	Val	Val	Lys	Ile
865					870					875					880
Ile	Gln	Pro	Leu	Asp	Arg	Glu	Val	Gln	Pro	Val	His	Tyr	Leu	Lys	Ile
			885						890					895	
Glu	Ala	Arg	Asp	Gln	Ala	Thr	Glu	Glu	Pro	Arg	Leu	Phe	Ser	Thr	Val
			900					905					910		
Leu	Leu	Lys	Val	Ser	Leu	Asp	Asp	Val	Asn	Asp	Asn	Pro	Pro	Arg	Phe
		915					920					925			
Ile	Pro	Pro	Asn	Tyr	Ser	Val	Lys	Val	Arg	Glu	Asp	Leu	Pro	Glu	Gly
	930						935				940				
Thr	Ile	Ile	Met	Trp	Leu	Glu	Ala	Tyr	Asp	Pro	Asp	Val	Gly	Gln	Ser
945					950					955					960
Ser	Gln	Val	Arg	Tyr	Ser	Leu	Leu	Asp	His	Gly	Glu	Gly	His	Phe	Asp
			965						970					975	
Val	Asp	Lys	Leu	Ser	Gly	Ala	Val	Arg	Ile	Val	Gln	Gln	Leu	Asp	Phe
		980						985					990		
Glu	Lys	Lys	Gln	Leu	Tyr	Asn	Leu	Thr	Val	Arg	Ala	Lys	Asp	Lys	Gly
		995					1000					1005			
Lys	Pro	Val	Ser	Leu	Ser	Ser	Thr	Cys	Tyr	Val	Glu	Val	Glu	Val	
	1010						1015				1020				
Val	Asp	Val	Asn	Glu	Asn	Leu	His	Thr	Pro	Val	Phe	Ser	Ser	Phe	
	1025						1030				1035				
Val	Glu	Lys	Gly	Val	Val	Lys	Glu	Asp	Val	Pro	Thr	Gly	Ser	Ser	
	1040						1045				1050				
Val	Met	Thr	Val	Ser	Ala	His	Asp	Glu	Asp	Thr	Gly	Arg	Asp	Gly	
	1055						1060				1065				
Glu	Ile	Arg	Tyr	Ser	Ile	Arg	Asp	Gly	Ser	Gly	Ile	Gly	Val	Phe	
	1070						1075				1080				
Arg	Ile	Asp	Glu	Glu	Thr	Gly	Val	Ile	Glu	Thr	Ser	Asp	Arg	Leu	
	1085						1090				1095				
Asp	Arg	Glu	Ser	Thr	Ser	His	Tyr	Trp	Leu	Thr	Val	Tyr	Ala	Thr	
	1100						1105				1110				

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Asp	Gln	Gly	Val	Val	Pro	Leu	Ser	Ser	Phe	Ile	Glu	Val	Tyr	Ile
1115						1120					1125			
Glu	Val	Glu	Asp	Val	Asn	Asp	Asn	Ala	Pro	Gln	Thr	Ser	Glu	Pro
1130						1135					1140			
Val	Tyr	Tyr	Pro	Glu	Ile	Met	Glu	Asn	Ser	Pro	Lys	Asp	Val	Ser
1145						1150					1155			
Val	Val	Gln	Ile	Glu	Ala	Phe	Asp	Pro	Asp	Ser	Ser	Ser	Asn	Asp
1160						1165					1170			
Lys	Leu	Thr	Tyr	Arg	Ile	Thr	Ser	Gly	Asn	Pro	Gln	Gly	Phe	Phe
1175						1180					1185			
Ser	Ile	His	Pro	Lys	Thr	Gly	Leu	Ile	Thr	Thr	Thr	Ser	Arg	Lys
1190						1195					1200			
Leu	Asp	Arg	Glu	Gln	Gln	Asp	Glu	His	Ile	Leu	Glu	Val	Thr	Val
1205						1210					1215			
Thr	Asp	Asn	Gly	Val	Pro	Pro	Arg	Ser	Thr	Ile	Ala	Arg	Val	Ile
1220						1225					1230			
Val	Lys	Ile	Leu	Asp	Glu	Asn	Asp	Asn	Arg	Pro	Gln	Phe	Leu	Gln
1235						1240					1245			
Lys	Phe	Tyr	Lys	Ile	Arg	Leu	Pro	Glu	Arg	Glu	Lys	Ala	Asp	Gly
1250						1255					1260			
Asp	Arg	Ser	Ala	Ser	Lys	Arg	Glu	Pro	Leu	Tyr	Arg	Val	Ile	Ala
1265						1270					1275			
Ala	Asp	Lys	Asp	Glu	Gly	Pro	Asn	Ala	Glu	Leu	Ser	Tyr	Ser	Ile
1280						1285					1290			
Glu	Glu	Gly	Asn	Glu	His	Gly	Arg	Phe	Ser	Ile	Glu	Pro	Lys	Thr
1295						1300					1305			
Gly	Val	Val	Ser	Ser	Lys	Lys	Phe	Ser	Ala	Ala	Gly	Glu	Tyr	Asp
1310						1315					1320			
Ile	Leu	Ser	Ile	Lys	Ala	Val	Asp	Asn	Gly	Arg	Pro	Gln	Lys	Ser
1325						1330					1335			
Ser	Thr	Thr	Arg	Leu	His	Ile	Glu	Trp	Ile	Ser	Lys	Pro	Lys	Pro
1340						1345					1350			
Ser	Ser	Glu	Pro	Ile	Ser	Phe	Glu	Glu	Ser	Val	Phe	Ser	Phe	Thr
1355						1360					1365			
Val	Met	Glu	Ser	Asp	Pro	Val	Ala	His	Met	Ile	Gly	Val	Ile	Ser
1370						1375					1380			
Val	Glu	Pro	Pro	Gly	Met	Pro	Leu	Trp	Phe	Asp	Ile	Ile	Gly	Gly
1385						1390					1395			
Asn	Tyr	Asp	Ser	His	Phe	Asp	Val	Asp	Lys	Gly	Thr	Gly	Thr	Ile
1400						1405					1410			
Ile	Val	Ala	Lys	Pro	Leu	Asp	Ala	Glu	Gln	Lys	Ser	Ser	Tyr	Asn
1415						1420					1425			
Leu	Thr	Val	Glu	Ala	Thr	Asp	Gly	Thr	Thr	Thr	Ile	Leu	Thr	Gln
1430						1435					1440			
Val	Leu	Ile	Lys	Val	Ile	Asp	Thr	Asn	Asp	His	Arg	Pro	Gln	Phe
1445						1450					1455			
Ser	Thr	Ser	Lys	Tyr	Glu	Val	Ala	Val	Pro	Glu	Asp	Thr	Glu	Pro
1460						1465					1470			
Glu	Val	Glu	Ile	Leu	Gln	Ile	Ser	Ala	Val	Asp	Arg	Asp	Glu	Lys
1475						1480					1485			
Asn	Lys	Leu	Ile	Tyr	Thr	Leu	Gln	Ser	Ser	Ile	Asp	Pro	Ala	Ser
1490						1495					1500			
Leu	Lys	Lys	Phe	Arg	Leu	Asp	Pro	Ala	Thr	Gly	Ala	Leu	Tyr	Thr
1505						1510					1515			

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Ala	Glu	Lys	Leu	Asp	His	Glu	Ala	Ile	His	Gln	His	Val	Leu	Thr
1520						1525					1530			
Val	Met	Val	Arg	Asp	Gln	Asp	Val	Pro	Val	Lys	Arg	Asn	Phe	Ala
1535						1540					1545			
Arg	Ile	Val	Val	Asn	Val	Ser	Asp	Lys	Asn	Asp	His	Ala	Pro	Trp
1550						1555					1560			
Phe	Thr	Ser	Pro	Ser	Tyr	Asp	Gly	Arg	Val	Tyr	Glu	Ser	Ala	Ala
1565						1570					1575			
Val	Gly	Ser	Val	Val	Leu	Gln	Val	Thr	Ala	Leu	Asp	Lys	Asp	Lys
1580						1585					1590			
Gly	Arg	Asn	Ala	Glu	Val	Leu	Tyr	Ser	Ile	Glu	Ser	Gly	Asn	Ile
1595						1600					1605			
Gly	Asn	Ser	Phe	Thr	Ile	Asp	Pro	Ile	Leu	Gly	Ser	Ile	Lys	Thr
1610						1615					1620			
Ala	Arg	Glu	Leu	Asp	Arg	Ser	His	Gln	Val	Asp	Tyr	Asp	Leu	Met
1625						1630					1635			
Val	Lys	Ala	Thr	Asp	Lys	Gly	Asp	Pro	Pro	Met	Ser	Glu	Met	Thr
1640						1645					1650			
Ser	Val	Arg	Ile	Ala	Val	Thr	Val	Ala	Asp	Asn	Ala	Ser	Pro	Lys
1655						1660					1665			
Phe	Thr	Ser	Lys	Glu	Tyr	Ser	Ala	Glu	Ile	Ser	Glu	Ala	Ile	Arg
1670						1675					1680			
Ile	Gly	Ser	Phe	Val	Gly	Met	Val	Ser	Ala	His	Ser	Gln	Ser	Ser
1685						1690					1695			
Val	Met	Tyr	Glu	Ile	Arg	Asp	Gly	Asn	Met	Gly	Asp	Ala	Phe	Asn
1700						1705					1710			
Ile	Asn	Pro	His	Ser	Gly	Ser	Ile	Ile	Thr	Gln	Arg	Ala	Leu	Asp
1715						1720					1725			
Phe	Glu	Thr	Leu	Pro	Met	Tyr	Ser	Leu	Thr	Val	Gln	Gly	Thr	Asn
1730						1735					1740			
Met	Ala	Gly	Leu	Ser	Thr	Asn	Thr	Thr	Val	Val	Val	His	Val	Arg
1745						1750					1755			
Asp	Glu	Asn	Asp	Asn	Pro	Pro	Val	Phe	Thr	Gln	Ala	Glu	Tyr	Ser
1760						1765					1770			
Gly	Phe	Ile	Ser	Glu	Ser	Ala	Ser	Val	Asn	Ser	Val	Val	Leu	Thr
1775						1780					1785			
Asp	Arg	Asn	Val	Pro	Leu	Val	Ile	Arg	Ala	Thr	Asp	Ala	Asp	Arg
1790						1795					1800			
Glu	Ser	Asn	Ala	Leu	Leu	Val	Tyr	Gln	Ile	Val	Glu	Pro	Ser	Val
1805						1810					1815			
His	Asn	Tyr	Phe	Ala	Ile	Asp	Pro	Thr	Thr	Gly	Ala	Ile	Arg	Thr
1820						1825					1830			
Val	Leu	Ser	Leu	Asp	Tyr	Glu	Glu	Thr	His	Ala	Phe	His	Phe	Thr
1835						1840					1845			
Val	Gln	Val	His	Asp	Met	Gly	Thr	Pro	Arg	Leu	Phe	Ala	Glu	Tyr
1850						1855					1860			
Ala	Ala	Asn	Val	Thr	Val	His	Val	Ile	Asp	Ile	Asn	Asp	Cys	Pro
1865						1870					1875			
Pro	Val	Phe	Ser	Lys	Ser	Leu	Tyr	Glu	Val	Ser	Leu	Leu	Leu	Pro
1880						1885					1890			
Thr	Tyr	Arg	Gly	Val	Asn	Val	Ile	Thr	Val	Asn	Ala	Thr	Asp	Ala
1895						1900					1905			
Asp	Ser	Lys	Ala	Phe	Ser	Gln	Val	Met	Tyr	Ser	Ile	Thr	Glu	Gly





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Ser	Glu	Pro	Asn	Arg	Gly	Ile	Ser	Tyr	Gln	Leu	Ile	Gly	Asn	His
2315						2320					2325			
Ser	Lys	Ser	His	Asp	His	Phe	His	Ile	Asp	Ser	Asn	Thr	Gly	Leu
2330						2335					2340			
Ile	Ser	Leu	Val	Arg	Ala	Leu	Asp	Tyr	Glu	Gln	Ser	Gln	Gln	His
2345						2350					2355			
Arg	Ile	Phe	Val	Arg	Ala	Val	Asp	Gly	Gly	Met	Pro	Ala	Leu	Ser
2360						2365					2370			
Ser	Asp	Val	Val	Val	Thr	Val	Ala	Val	Thr	Asp	Leu	Asn	Asp	Asn
2375						2380					2385			
Pro	Pro	Leu	Phe	Glu	Gln	Gln	Val	Tyr	Glu	Ala	Arg	Ile	Ser	Glu
2390						2395					2400			
His	Ala	Ala	His	Gly	His	Phe	Val	Met	Cys	Val	Arg	Ala	Cys	Asp
2405						2410					2415			
Ala	Asp	Ser	Ser	Asp	Leu	Asp	Lys	Leu	Glu	Tyr	Ser	Ile	Leu	Ser
2420						2425					2430			
Gly	Asn	Asp	His	Lys	Ser	Phe	Ile	Ile	Asp	Arg	Glu	Thr	Gly	Ile
2435						2440					2445			
Ile	Thr	Leu	Ser	Asn	Leu	Arg	Arg	His	Thr	Leu	Lys	Pro	Phe	Tyr
2450						2455					2460			
Ser	Leu	Asn	Val	Ser	Val	Ser	Asp	Gly	Val	Phe	Arg	Ser	Ser	Ala
2465						2470					2475			
Arg	Val	Asn	Val	Thr	Val	Met	Gly	Gly	Asn	Leu	His	Ser	Pro	Val
2480						2485					2490			
Phe	His	Gln	Asn	Glu	Tyr	Glu	Val	Glu	Leu	Ala	Glu	Asn	Ala	Pro
2495						2500					2505			
Leu	His	Thr	Leu	Val	Val	Gln	Val	Lys	Ala	Ser	Asp	Arg	Asp	Ser
2510						2515					2520			
Gly	Ile	Tyr	Ser	His	Val	Thr	Tyr	His	Ile	Val	Asn	Asp	Phe	Ala
2525						2530					2535			
Lys	Asp	Arg	Phe	Tyr	Val	Asn	Asp	Arg	Gly	Gln	Ile	Phe	Thr	Leu
2540						2545					2550			
Glu	Lys	Leu	Asp	Arg	Glu	Thr	Pro	Ala	Glu	Lys	Val	Ile	Ser	Ile
2555						2560					2565			
Arg	Leu	Met	Ala	Lys	Asp	Ala	Gly	Gly	Lys	Val	Ala	Phe	Cys	Thr
2570						2575					2580			
Val	Asn	Val	Ile	Leu	Thr	Asp	Asp	Asn	Asp	Asn	Ala	Pro	Gln	Phe
2585						2590					2595			
Arg	Ser	Thr	Lys	Tyr	Glu	Val	Asn	Ile	Gly	Ser	Ser	Ala	Ala	Lys
2600						2605					2610			
Gly	Thr	Ser	Val	Val	Lys	Val	Phe	Ala	Ser	Asp	Ala	Asp	Glu	Gly
2615						2620					2625			
Ser	Asn	Ala	Asp	Val	Thr	Tyr	Ala	Ile	Glu	Ala	Asp	Ser	Glu	Ser
2630						2635					2640			
Val	Lys	Glu	Asn	Leu	Glu	Ile	Asn	Lys	Leu	Thr	Gly	Leu	Ile	Thr
2645						2650					2655			
Thr	Lys	Glu	Ser	Leu	Ile	Gly	Leu	Glu	Asn	Glu	Phe	Phe	Thr	Phe
2660						2665					2670			
Phe	Val	Arg	Ala	Val	Asp	Ser	Gly	Ser	Pro	Pro	Arg	Glu	Ser	Val
2675						2680					2685			
Val	Pro	Val	Tyr	Ile	Lys	Ile	Leu	Pro	Pro	Glu	Val	Gln	Leu	Pro
2690						2695					2700			
Arg	Phe	Ser	Glu	Pro	Phe	Tyr	Thr	Tyr	Thr	Ile	Ser	Glu	Asp	Thr
2705						2710					2715			

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Pro	Ile	Gly	Thr	Glu	Ile	Asp	Leu	Ile	Arg	Val	Glu	His	Gly	Gly
	2720					2725					2730			
Ala	Val	Leu	Tyr	Ile	Leu	Val	Lys	Gly	Asn	Thr	Pro	Glu	Ser	Asn
	2735					2740					2745			
Arg	Asp	Glu	Phe	Phe	Val	Ile	Asp	Arg	Gln	Asn	Gly	Arg	Leu	Lys
	2750					2755					2760			
Leu	Glu	Lys	Ser	Leu	Asp	His	Glu	Thr	Thr	Lys	Trp	Tyr	Gln	Phe
	2765					2770					2775			
Ser	Ile	Leu	Ala	Arg	Cys	Thr	Leu	Asp	Asp	Tyr	Glu	Val	Val	Ala
	2780					2785					2790			
Ser	Ile	Asp	Val	Ser	Ile	Gln	Val	Lys	Asp	Ala	Asn	Asp	Asn	Ser
	2795					2800					2805			
Pro	Val	Leu	Glu	Ser	Ser	Pro	Tyr	Glu	Ala	Phe	Ile	Val	Glu	Asn
	2810					2815					2820			
Leu	Pro	Gly	Gly	Ser	Arg	Val	Ile	Gln	Ile	Arg	Ala	Ser	Asp	Leu
	2825					2830					2835			
Asp	Ser	Gly	Ala	Asn	Gly	Gln	Val	Met	Tyr	Ser	Leu	Asp	Gln	Ser
	2840					2845					2850			
Gln	Asp	Ala	Asp	Ile	Ile	Glu	Ser	Phe	Ala	Ile	Asn	Met	Glu	Thr
	2855					2860					2865			
Gly	Trp	Ile	Thr	Thr	Leu	Lys	Glu	Leu	Asp	His	Glu	Glu	Arg	Ala
	2870					2875					2880			
Ser	Tyr	Gln	Ile	Lys	Val	Val	Ala	Ser	Asp	His	Gly	Glu	Lys	Val
	2885					2890					2895			
Gln	Leu	Ser	Ser	Thr	Ala	Ile	Val	Gly	Val	Thr	Val	Thr	Asp	Val
	2900					2905					2910			
Asn	Asp	Ser	Pro	Pro	Arg	Phe	Thr	Ala	Glu	Ile	Tyr	Lys	Gly	Thr
	2915					2920					2925			
Val	Ser	Glu	Asp	Asp	Pro	Pro	Gly	Gly	Val	Ile	Ala	Ile	Leu	Ser
	2930					2935					2940			
Thr	Thr	Asp	Ala	Asp	Thr	Glu	Glu	Ile	Asn	Arg	Gln	Val	Ser	Tyr
	2945					2950					2955			
Phe	Ile	Thr	Gly	Gly	Asp	Ala	Leu	Gly	Gln	Phe	Ala	Val	Glu	Asn
	2960					2965					2970			
Val	Gln	Ser	Asp	Trp	Arg	Val	Tyr	Val	Lys	Lys	Pro	Leu	Asp	Arg
	2975					2980					2985			
Glu	Gln	Lys	Asp	Ser	Tyr	Leu	Leu	Thr	Val	Thr	Ala	Thr	Asp	Gly
	2990					2995					3000			
Thr	Phe	Ser	Ser	Lys	Ala	Arg	Val	Glu	Val	Lys	Val	Leu	Asp	Ala
	3005					3010					3015			
Asn	Asp	Asn	Ser	Pro	Val	Cys	Glu	Lys	Thr	Ser	Tyr	Ser	Asp	Thr
	3020					3025					3030			
Ile	Pro	Glu	Asp	Ala	Leu	Pro	Gly	Lys	Leu	Val	Met	Gln	Val	Ser
	3035					3040					3045			
Ala	Thr	Asp	Ala	Asp	Ile	Arg	Ser	Asn	Ala	Glu	Ile	Thr	Tyr	Thr
	3050					3055					3060			
Leu	Phe	Gly	Ser	Gly	Ala	Glu	Lys	Phe	Lys	Leu	Asn	Pro	Asp	Thr
	3065					3070					3075			
Gly	Glu	Leu	Arg	Thr	Leu	Ala	Leu	Leu	Asp	Arg	Glu	Glu	Gln	Ala
	3080					3085					3090			
Val	Tyr	Asn	Leu	Leu	Val	Lys	Ala	Thr	Asp	Gly	Gly	Gly	Arg	Ser
	3095					3100					3105			
Cys	Gln	Ala	Ala	Ile	Val	Leu	Thr	Leu	Glu	Asp	Val	Asn	Asp	Asn

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3110	3115	3120
Ala Pro Glu Phe Thr Ala Glu Pro Tyr Thr Ile Thr Val Phe Glu 3125	3130	3135
Asn Thr Glu Pro Gly Thr Pro Leu Thr Arg Val Gln Ala Thr Asp 3140	3145	3150
Ala Asp Thr Gly Leu Asn Arg Lys Ile Ser Tyr Ser Leu Val Glu 3155	3160	3165
Ser Ala Asp Gly Gln Phe Ser Ile Asn Glu Arg Ser Gly Ile Ile 3170	3175	3180
Gln Leu Glu Lys His Leu Asp Arg Glu Leu Gln Ala Val Tyr Thr 3185	3190	3195
Leu Thr Leu Lys Ala Val Asp Gln Gly Leu Pro Arg Arg Leu Thr 3200	3205	3210
Ala Thr Gly Thr Val Val Val Ser Val Leu Asp Ile Asn Asp Asn 3215	3220	3225
Pro Pro Val Phe Glu Tyr Arg Glu Tyr Gly Ala Ser Val Ser Glu 3230	3235	3240
Asp Ile Val Ile Gly Thr Glu Val Leu Gln Val Tyr Ala Ala Ser 3245	3250	3255
Arg Asp Ile Glu Ala Asn Ala Glu Ile Thr Tyr Ala Ile Ile Ser 3260	3265	3270
Gly Asn Glu His Gly Lys Phe Ser Ile Asp Ser Lys Thr Gly Ala 3275	3280	3285
Ile Phe Ile Ile Glu Ser Leu Asp Tyr Glu Ser Ser His Glu Tyr 3290	3295	3300
Tyr Leu Thr Val Glu Ala Thr Asp Gly Gly Thr Pro Ser Leu Ser 3305	3310	3315
Asp Val Ala Thr Val Asn Ile Asn Val Thr Asp Ile Asn Asp Asn 3320	3325	3330
Ser Pro Val Phe Ser Gln Asp Thr Tyr Thr Thr Val Val Ser Glu 3335	3340	3345
Asp Ala Ala Leu Glu Gln Pro Val Ile Thr Ile Met Ala Asp Asp 3350	3355	3360
Ala Asp Gly Pro Ser Asn Ser His Ile His Tyr Ser Ile Ile Glu 3365	3370	3375
Gly Asn Gln Gly Ser Pro Phe Thr Ile Asp Pro Val Arg Gly Glu 3380	3385	3390
Val Lys Val Thr Lys Pro Leu Asp Arg Glu Thr Ile Ser Gly Tyr 3395	3400	3405
Thr Leu Thr Val Gln Ala Ala Asp Asn Gly Asn Pro Pro Arg Val 3410	3415	3420
Asn Thr Thr Thr Val Asn Ile Asp Val Ser Asp Val Asn Asp Asn 3425	3430	3435
Ala Pro Leu Phe Ser Arg Asp Asn Tyr Ser Val Ile Ile Gln Glu 3440	3445	3450
Asn Lys Pro Val Gly Phe Ser Val Leu Lys Leu Val Val Thr Asp 3455	3460	3465
Lys Asp Ser Ser His Asn Gly Pro Pro Phe Phe Phe Thr Ile Val 3470	3475	3480
Ser Gly Asn Asp Glu Asn Ala Phe Glu Val Asn Gln His Gly Val 3485	3490	3495
Leu Leu Thr Ala Ala Thr Ile Lys Arg Lys Val Lys Asp His Tyr 3500	3505	3510

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Leu	Leu	His	Val	Lys	Val	Ala	Asp	Ser	Gly	Lys	Pro	Gln	Leu	Ser
3515						3520					3525			
Ser	Met	Thr	His	Ile	Asp	Ile	Arg	Val	Ile	Glu	Glu	Ser	Ile	His
3530						3535					3540			
Pro	Pro	Ala	Ile	Leu	Pro	Leu	Glu	Ile	Phe	Ile	Thr	Ala	Phe	Gly
3545						3550					3555			
Glu	Glu	Tyr	Ser	Gly	Gly	Val	Ile	Gly	Lys	Ile	His	Ala	Thr	Asp
3560						3565					3570			
Gln	Asp	Val	Tyr	Asp	Thr	Leu	Met	Tyr	Ser	Leu	Asp	Pro	His	Met
3575						3580					3585			
Asp	Gly	Leu	Phe	Ser	Val	Ser	Ser	Thr	Gly	Gly	Lys	Leu	Ile	Ala
3590						3595					3600			
His	Arg	Lys	Leu	Asp	Ile	Gly	Gln	Tyr	Leu	Leu	Asn	Val	Ser	Val
3605						3610					3615			
Thr	Asp	Gly	Lys	Phe	Thr	Thr	Val	Ala	Asp	Ile	Thr	Val	His	Ile
3620						3625					3630			
Gln	Gln	Val	Thr	Gln	Glu	Met	Leu	Asn	His	Thr	Val	Ala	Ile	Arg
3635						3640					3645			
Phe	Ala	Asn	Leu	Thr	Pro	Glu	Glu	Phe	Val	Gly	Asp	Tyr	Trp	Arg
3650						3655					3660			
Asn	Phe	Gln	Arg	Ala	Leu	Arg	Asn	Ile	Leu	Gly	Val	Arg	Lys	Asn
3665						3670					3675			
Asp	Ile	Gln	Ile	Val	Ser	Leu	Gln	Pro	Ser	Glu	Pro	His	Ser	His
3680						3685					3690			
Leu	Asp	Val	Leu	Leu	Phe	Val	Glu	Arg	Ser	Gly	Gly	Thr	His	Val
3695						3700					3705			
Ser	Thr	Lys	Gln	Leu	Leu	His	Lys	Ile	Asn	Ser	Ser	Val	Thr	Asp
3710						3715					3720			
Val	Glu	Glu	Ile	Ile	Gly	Val	Arg	Ile	Leu	Glu	Val	Phe	Gln	Lys
3725						3730					3735			
Leu	Cys	Ala	Gly	Leu	Asp	Cys	Pro	Trp	Lys	Phe	Cys	Asp	Glu	Lys
3740						3745					3750			
Val	Ser	Val	Asp	Glu	Asn	Val	Met	Ser	Thr	His	Ser	Thr	Ala	Arg
3755						3760					3765			
Leu	Ser	Phe	Val	Thr	Pro	Arg	His	His	Arg	Thr	Ala	Val	Cys	Leu
3770						3775					3780			
Cys	Lys	Asp	Gly	Thr	Cys	Pro	Pro	Val	His	His	Gly	Cys	Glu	Asp
3785						3790					3795			
Asn	Pro	Cys	Pro	Ala	Gly	Ser	Glu	Cys	Val	Ala	Asp	Pro	Arg	Glu
3800						3805					3810			
Glu	Lys	Tyr	Ser	Cys	Val	Cys	Pro	Gly	Gly	Gly	Phe	Gly	Lys	Cys
3815						3820					3825			
Pro	Gly	Ser	Ser	Ser	Ile	Thr	Phe	Thr	Gly	Asn	Ser	Phe	Val	Lys
3830						3835					3840			
Tyr	Arg	Leu	Leu	Glu	Asn	Glu	Asn	Arg	Leu	Glu	Met	Lys	Leu	Ser
3845						3850					3855			
Met	Arg	Leu	Arg	Thr	Tyr	Ser	Ser	His	Ala	Val	Val	Met	Tyr	Ala
3860						3865					3870			
Arg	Gly	Thr	Asp	Tyr	Ser	Ile	Pro	Gly	Ile	Val	Ser	Val	Gln	Ser
3875						3880					3885			
Ile	Gln	Val	Asn	Asp	Gly	Gln	Trp	His	Ala	Val	Ser	Leu	Glu	Val
3890						3895					3900			
Glu	Gly	Asn	Tyr	Ala	Lys	Leu	Val	Leu	Asp	Glu	Val	His	Thr	Ala
3905						3910					3915			

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Ser Gly	Thr Ala	Pro Gly	Ala Leu	Lys Thr	Leu Asn	Leu Asp	Asn						
3920			3925		3930								
Tyr Val	Phe Phe	Gly Gly	His Leu	Arg Gln	Gln Gly	Thr Lys	His						
3935			3940		3945								
Gly Arg	Gly Ala	Gln Val	Ala Ser	Gly Phe	Arg Gly	Cys Met	Asp						
3950			3955		3960								
Ser Ile	Tyr Leu	Asn Gly	Gln Glu	Leu Pro	Leu Asn	Asn Lys	Pro						
3965			3970		3975								
Arg Ala	Tyr Ala	His Ile	Glu Glu	Trp Val	Asp Leu	Ser His	Gly						
3980			3985		3990								
Cys Leu	Leu Thr	Ala Thr	Glu Asp	Cys Ser	Ser Ser	Pro Cys	Gln						
3995			4000		4005								
Asn Gly	Gly Val	Cys Asn	Pro Ser	Pro Thr	Gly Gly	Tyr Tyr	Cys						
4010			4015		4020								
Lys Cys	Ser Ala	Leu Tyr	Val Gly	Thr Phe	Cys Glu	Val Ser	Val						
4025			4030		4035								
Asn Pro	Cys Ser	Ser Asn	Pro Cys	Leu Tyr	Gly Gly	Thr Cys	Met						
4040			4045		4050								
Val Asp	Asn Gly	Gly Phe	Val Cys	Gln Cys	Arg Gly	Leu Tyr	Thr						
4055			4060		4065								
Gly Leu	Val Leu	Phe Phe	Ser Phe	Cys Met	Cys Phe	Ser Leu	Phe						
4070			4075		4080								
Arg Cys	Gln Leu	Ser Pro	Tyr Cys	Lys Asp	Asp Pro	Cys Lys	Asn						
4085			4090		4095								
Gly Gly	Thr Cys	Phe Asp	Ser Leu	Asp Gly	Ala Val	Cys Gln	Cys						
4100			4105		4110								
Asp Ser	Gly Phe	Arg Gly	Glu Arg	Cys Gln	Ser Asp	Ile Asp	Glu						
4115			4120		4125								
Cys Ala	Gly Asn	Pro Cys	Arg Asn	Gly Ala	Leu Cys	Glu Asn	Thr						
4130			4135		4140								
His Gly	Ser Tyr	His Cys	Asn Cys	Ser Gln	Glu Tyr	Arg Gly	Lys						
4145			4150		4155								
His Cys	Glu Asp	Ala Ser	Pro Asn	His Tyr	Val Ser	Thr Pro	Trp						
4160			4165		4170								
Asn Ile	Gly Leu	Ala Glu	Gly Ile	Gly Ile	Ile Val	Phe Ile	Ala						
4175			4180		4185								
Gly Ile	Val Leu	Leu Val	Met Val	Phe Val	Leu Cys	Arg Lys	Met						
4190			4195		4200								
Ile Ser	Arg Lys	Lys Lys	Arg Gln	Ala Glu	Pro Glu	Asp Lys	Arg						
4205			4210		4215								
Leu Gly	Pro Thr	Thr Ala	Phe Leu	Gln Arg	Pro Tyr	Phe Asp	Ser						
4220			4225		4230								
Lys Leu	Asn Lys	Asn Ile	Tyr Ser	Asp Ile	Pro Pro	Gln Val	Pro						
4235			4240		4245								
Val Arg	Pro Ile	Ser Tyr	Thr Pro	Ser Ile	Pro Ser	Asp Ser	Arg						
4250			4255		4260								
Asn Asn	Leu Asp	Arg Asn	Ser Phe	Glu Gly	Ser Ala	Ile Pro	Glu						
4265			4270		4275								
His Pro	Glu Phe	Ser Thr	Phe Asn	Pro Glu	Ser Met	His Gly	His						
4280			4285		4290								
Arg Lys	Ala Val	Ala Val	Cys Ser	Val Ala	Pro Asn	Leu Pro	Pro						
4295			4300		4305								
Pro Pro	Pro Ser	Asn Ser	Pro Ser	Asp Ser	Asp Ser	Ile Gln	Lys						

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4310	4315	4320
Pro Ser Trp Asp Phe Asp Tyr Asp Ala Lys Val Val Asp Leu Asp		
4325	4330	4335
Pro Cys Leu Ser Lys Lys Pro Leu Glu Glu Lys Pro Ser Gln Pro		
4340	4345	4350
Tyr Ser Ala Arg Glu Ser Leu Ser Glu Val Gln Ser Leu Ser Ser		
4355	4360	4365
Phe Gln Ser Glu Ser Cys Asp Asp Asn Glu Ser Leu Ala Ala Pro		
4370	4375	4380
Asp Leu Ser Lys Pro Arg Gly Tyr His Trp Asp Thr Ser Asp Trp		
4385	4390	4395
Met Pro Ser Val Pro Leu Pro Asp Ile Gln Glu Phe Pro Asn Tyr		
4400	4405	4410
Glu Ala Ile Asp Glu His Thr Pro Leu Tyr Ser Ala Asp Pro Asn		
4415	4420	4425
Ala Ile Asp Thr Asp Tyr Tyr Pro Gly Gly Tyr Asp Ile Glu Ser		
4430	4435	4440
Asp Phe Pro Pro Pro Pro Glu Asp Phe Pro Ala Pro Asp Glu Leu		
4445	4450	4455
Pro Pro Leu Pro Pro Glu Phe Ser Asp Gln Phe Glu Ser Ile His		
4460	4465	4470
Pro Pro Arg Asp Met Pro Ala Ala Gly Ser Leu Gly Ser Ser Ser		
4475	4480	4485
Arg Ser Arg Gln Arg Phe Asn Leu Asn Gln Tyr Leu Pro Asn Phe		
4490	4495	4500
Tyr Pro Ala Asp Met Ser Glu Pro Gln Lys Gln Gly Ala Gly Glu		
4505	4510	4515
Asn Ser Pro Cys Arg Glu Pro Tyr Thr Pro Tyr Pro Pro Gly Tyr		
4520	4525	4530
Gln Arg Asn Phe Glu Ala Pro Thr Ile Glu Asn Met Pro Met Ser		
4535	4540	4545
Val Tyr Ala Ser Thr Ala Ser Cys Ser Asp Val Ser Ala Cys Cys		
4550	4555	4560
Glu Val Glu Ser Glu Val Met Met Ser Asp Tyr Glu Ser Gly Asp		
4565	4570	4575
Asp Gly His Phe Glu Glu Val Thr Ile Pro Pro Leu Asp Ser Gln		
4580	4585	4590
Gln His Thr Glu Val		
4595		

&lt;210&gt; SEQ ID NO 2

&lt;211&gt; LENGTH: 4588

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 2

Met Gly Arg His Leu Ala Leu Leu Leu Leu Leu Leu Leu Phe Gln		
1	5	10 15
His Phe Gly Asp Ser Asp Gly Ser Gln Arg Leu Glu Gln Thr Pro Leu		
	20	25 30
Gln Phe Thr His Leu Glu Tyr Asn Val Thr Val Gln Glu Asn Ser Ala		
	35	40 45
Ala Lys Thr Tyr Val Gly His Pro Val Lys Met Gly Val Tyr Ile Thr		
	50	55 60
His Pro Ala Trp Glu Val Arg Tyr Lys Ile Val Ser Gly Asp Ser Glu		

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65	70	75	80
Asn Leu Phe Lys	Ala Glu Glu Tyr Ile	Leu Gly Asp Phe Cys Phe Leu	
	85	90	95
Arg Ile Arg Thr	Lys Gly Gly Asn Thr Ala Ile Leu Asn Arg Glu Val		
	100	105	110
Lys Asp His Tyr	Thr Leu Ile Val Lys Ala Leu Glu Lys Asn Thr Asn		
	115	120	125
Val Glu Ala Arg	Thr Lys Val Arg Val Gln Val Leu Asp Thr Asn Asp		
	130	135	140
Leu Arg Pro Leu	Phe Ser Pro Thr Ser Tyr Ser Val Ser Leu Pro Glu		
	145	150	155
Asn Thr Ala Ile	Arg Thr Ser Ile Ala Arg Val Ser Ala Thr Asp Ala		
	165	170	175
Asp Ile Gly Thr	Asn Gly Glu Phe Tyr Tyr Ser Phe Lys Asp Arg Thr		
	180	185	190
Asp Met Phe Ala	Ile His Pro Thr Ser Gly Val Ile Val Leu Thr Gly		
	195	200	205
Arg Leu Asp Tyr	Leu Glu Thr Lys Leu Tyr Glu Met Glu Ile Leu Ala		
	210	215	220
Ala Asp Arg Gly	Met Lys Leu Tyr Gly Ser Ser Gly Ile Ser Ser Met		
	225	230	235
Ala Lys Leu Thr	Val His Ile Glu Gln Ala Asn Glu Cys Ala Pro Val		
	245	250	255
Ile Thr Ala Val	Thr Leu Ser Pro Ser Glu Leu Asp Arg Asp Pro Ala		
	260	265	270
Tyr Ala Ile Val	Thr Val Asp Asp Cys Asp Gln Gly Ala Asn Gly Asp		
	275	280	285
Ile Ala Ser Leu	Ser Ile Val Ala Gly Asp Leu Leu Gln Gln Phe Arg		
	290	295	300
Thr Val Arg Ser	Phe Pro Gly Ser Lys Glu Tyr Lys Val Lys Ala Ile		
	305	310	315
Gly Gly Ile Asp	Trp Asp Ser His Pro Phe Gly Tyr Asn Leu Thr Leu		
	325	330	335
Gln Ala Lys Asp	Lys Gly Thr Pro Pro Gln Phe Ser Ser Val Lys Val		
	340	345	350
Ile His Val Thr	Ser Pro Gln Phe Lys Ala Gly Pro Val Lys Phe Glu		
	355	360	365
Lys Asp Val Tyr	Arg Ala Glu Ile Ser Glu Phe Ala Pro Pro Asn Thr		
	370	375	380
Pro Val Val Met	Val Lys Ala Ile Pro Ala Tyr Ser His Leu Arg Tyr		
	385	390	395
Val Phe Lys Ser	Thr Pro Gly Lys Ala Lys Phe Ser Leu Asn Tyr Asn		
	405	410	415
Thr Gly Leu Ile	Ser Ile Leu Glu Pro Val Lys Arg Gln Gln Ala Ala		
	420	425	430
His Phe Glu Leu	Glu Val Thr Thr Ser Asp Arg Lys Ala Ser Thr Lys		
	435	440	445
Val Leu Val Lys	Val Leu Gly Ala Asn Ser Asn Pro Pro Glu Phe Thr		
	450	455	460
Gln Thr Ala Tyr	Lys Ala Ala Phe Asp Glu Asn Val Pro Ile Gly Thr		
	465	470	475
Thr Val Met Ser	Leu Ser Ala Val Asp Pro Asp Glu Gly Glu Asn Gly		
	485	490	495



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Tyr Val Thr Tyr Ser Ile Ala Asn Leu Asn His Val Pro Phe Ala Ile  
                   500                  505                  510

Asp His Phe Thr Gly Ala Val Ser Thr Ser Glu Asn Leu Asp Tyr Glu  
           515                  520                  525

Leu Met Pro Arg Val Tyr Thr Leu Arg Ile Arg Ala Ser Asp Trp Gly  
       530                  535                  540

Leu Pro Tyr Arg Arg Glu Val Glu Val Leu Ala Thr Ile Thr Leu Asn  
 545                  550                  555                  560

Asn Leu Asn Asp Asn Thr Pro Leu Phe Glu Lys Ile Asn Cys Glu Gly  
                   565                  570                  575

Thr Ile Pro Arg Asp Leu Gly Val Gly Glu Gln Ile Thr Thr Val Ser  
           580                  585                  590

Ala Ile Asp Ala Asp Glu Leu Gln Leu Val Gln Tyr Gln Ile Glu Ala  
           595                  600                  605

Gly Asn Glu Leu Asp Phe Phe Ser Leu Asn Pro Asn Ser Gly Val Leu  
       610                  615                  620

Ser Leu Lys Arg Ser Leu Met Asp Gly Leu Gly Ala Lys Val Ser Phe  
 625                  630                  635                  640

His Ser Leu Arg Ile Thr Ala Thr Asp Gly Glu Asn Phe Ala Thr Pro  
           645                  650                  655

Leu Tyr Ile Asn Ile Thr Val Ala Ala Ser His Lys Leu Val Asn Leu  
           660                  665                  670

Gln Cys Glu Glu Thr Gly Val Ala Lys Met Leu Ala Glu Lys Leu Leu  
       675                  680                  685

Gln Ala Asn Lys Leu His Asn Gln Gly Glu Val Glu Asp Ile Phe Phe  
       690                  695                  700

Asp Ser His Ser Val Asn Ala His Ile Pro Gln Phe Arg Ser Thr Leu  
 705                  710                  715                  720

Pro Thr Gly Ile Gln Val Lys Glu Asn Gln Pro Val Gly Ser Ser Val  
           725                  730                  735

Ile Phe Met Asn Ser Thr Asp Leu Asp Thr Gly Phe Asn Gly Lys Leu  
           740                  745                  750

Val Tyr Ala Val Ser Gly Gly Asn Glu Asp Ser Cys Phe Met Ile Asp  
       755                  760                  765

Met Glu Thr Gly Met Leu Lys Ile Leu Ser Pro Leu Asp Arg Glu Thr  
       770                  775                  780

Thr Asp Lys Tyr Thr Leu Asn Ile Thr Val Tyr Asp Leu Gly Ile Pro  
 785                  790                  795                  800

Gln Lys Ala Ala Trp Arg Leu Leu His Val Val Val Val Asp Ala Asn  
           805                  810                  815

Asp Asn Pro Pro Glu Phe Leu Gln Glu Ser Tyr Phe Val Glu Val Ser  
       820                  825                  830

Glu Asp Lys Glu Val His Ser Glu Ile Ile Gln Val Glu Ala Thr Asp  
       835                  840                  845

Lys Asp Leu Gly Pro Asn Gly His Val Thr Tyr Ser Ile Val Thr Asp  
       850                  855                  860

Thr Asp Thr Phe Ser Ile Asp Ser Val Thr Gly Val Val Asn Ile Ala  
 865                  870                  875                  880

Arg Pro Leu Asp Arg Glu Leu Gln His Glu His Ser Leu Lys Ile Glu  
       885                  890                  895

Ala Arg Asp Gln Ala Arg Glu Glu Pro Gln Leu Phe Ser Thr Val Val  
       900                  905                  910

Val Lys Val Ser Leu Glu Asp Val Asn Asp Asn Pro Pro Thr Phe Ile  
       915                  920                  925

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Pro Pro Asn Tyr Arg Val Lys Val Arg Glu Asp Leu Pro Glu Gly Thr  
 930 935 940

Val Ile Met Trp Leu Glu Ala His Asp Pro Asp Leu Gly Gln Ser Gly  
 945 950 955 960

Gln Val Arg Tyr Ser Leu Leu Asp His Gly Glu Gly Asn Phe Asp Val  
 965 970 975

Asp Lys Leu Ser Gly Ala Val Arg Ile Val Gln Gln Leu Asp Phe Glu  
 980 985 990

Lys Lys Gln Val Tyr Asn Leu Thr Val Arg Ala Lys Asp Lys Gly Lys  
 995 1000 1005

Pro Val Ser Leu Ser Ser Thr Cys Tyr Val Glu Val Glu Val Val  
 1010 1015 1020

Asp Val Asn Glu Asn Leu His Pro Pro Val Phe Ser Ser Phe Val  
 1025 1030 1035

Glu Lys Gly Thr Val Lys Glu Asp Ala Pro Val Gly Ser Leu Val  
 1040 1045 1050

Met Thr Val Ser Ala His Asp Glu Asp Ala Arg Arg Asp Gly Glu  
 1055 1060 1065

Ile Arg Tyr Ser Ile Arg Asp Gly Ser Gly Val Gly Val Phe Lys  
 1070 1075 1080

Ile Gly Glu Glu Thr Gly Val Ile Glu Thr Ser Asp Arg Leu Asp  
 1085 1090 1095

Arg Glu Ser Thr Ser His Tyr Trp Leu Thr Val Phe Ala Thr Asp  
 1100 1105 1110

Gln Gly Val Val Pro Leu Ser Ser Phe Ile Glu Ile Tyr Ile Glu  
 1115 1120 1125

Val Glu Asp Val Asn Asp Asn Ala Pro Gln Thr Ser Glu Pro Val  
 1130 1135 1140

Tyr Tyr Pro Glu Ile Met Glu Asn Ser Pro Lys Asp Val Ser Val  
 1145 1150 1155

Val Gln Ile Glu Ala Phe Asp Pro Asp Ser Ser Ser Asn Asp Lys  
 1160 1165 1170

Leu Met Tyr Lys Ile Thr Ser Gly Asn Pro Gln Gly Phe Phe Ser  
 1175 1180 1185

Ile His Pro Lys Thr Gly Leu Ile Thr Thr Thr Ser Arg Lys Leu  
 1190 1195 1200

Asp Arg Glu Gln Gln Asp Glu His Ile Leu Glu Val Thr Val Thr  
 1205 1210 1215

Asp Asn Gly Ser Pro Pro Lys Ser Thr Ile Ala Arg Val Ile Val  
 1220 1225 1230

Lys Ile Leu Asp Glu Asn Asp Asn Lys Pro Gln Phe Leu Gln Lys  
 1235 1240 1245

Phe Tyr Lys Ile Arg Leu Pro Glu Arg Glu Lys Pro Asp Arg Glu  
 1250 1255 1260

Arg Asn Ala Arg Arg Glu Pro Leu Tyr His Val Ile Ala Thr Asp  
 1265 1270 1275

Lys Asp Glu Gly Pro Asn Ala Glu Ile Ser Tyr Ser Ile Glu Asp  
 1280 1285 1290

Gly Asn Glu His Gly Lys Phe Phe Ile Glu Pro Lys Thr Gly Val  
 1295 1300 1305

Val Ser Ser Lys Arg Phe Ser Ala Ala Gly Glu Tyr Asp Ile Leu  
 1310 1315 1320

Ser Ile Lys Ala Val Asp Asn Gly Arg Pro Gln Lys Ser Ser Thr

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1325	1330	1335
Thr Arg Leu His Ile Glu Trp Ile Ser Lys Pro Lys Pro Ser Leu 1340	1345	1350
Glu Pro Ile Ser Phe Glu Glu Ser Phe Phe Thr Phe Thr Val Met 1355	1360	1365
Glu Ser Asp Pro Val Ala His Met Ile Gly Val Ile Ser Val Glu 1370	1375	1380
Pro Pro Gly Ile Pro Leu Trp Phe Asp Ile Thr Gly Gly Asn Tyr 1385	1390	1395
Asp Ser His Phe Asp Val Asp Lys Gly Thr Gly Thr Ile Ile Val 1400	1405	1410
Ala Lys Pro Leu Asp Ala Glu Gln Lys Ser Asn Tyr Asn Leu Thr 1415	1420	1425
Val Glu Ala Thr Asp Gly Thr Thr Thr Ile Leu Thr Gln Val Phe 1430	1435	1440
Ile Lys Val Ile Asp Thr Asn Asp His Arg Pro Gln Phe Ser Thr 1445	1450	1455
Ser Lys Tyr Glu Val Val Ile Pro Glu Asp Thr Ala Pro Glu Thr 1460	1465	1470
Glu Ile Leu Gln Ile Ser Ala Val Asp Gln Asp Glu Lys Asn Lys 1475	1480	1485
Leu Ile Tyr Thr Leu Gln Ser Ser Arg Asp Pro Leu Ser Leu Lys 1490	1495	1500
Lys Phe Arg Leu Asp Pro Ala Thr Gly Ser Leu Tyr Thr Ser Glu 1505	1510	1515
Lys Leu Asp His Glu Ala Val His Gln His Thr Leu Thr Val Met 1520	1525	1530
Val Arg Asp Gln Asp Val Pro Val Lys Arg Asn Phe Ala Arg Ile 1535	1540	1545
Val Val Asn Val Ser Asp Thr Asn Asp His Ala Pro Trp Phe Thr 1550	1555	1560
Ala Ser Ser Tyr Lys Gly Arg Val Tyr Glu Ser Ala Ala Val Gly 1565	1570	1575
Ser Val Val Leu Gln Val Thr Ala Leu Asp Lys Asp Lys Gly Lys 1580	1585	1590
Asn Ala Glu Val Leu Tyr Ser Ile Glu Ser Gly Asn Ile Gly Asn 1595	1600	1605
Ser Phe Met Ile Asp Pro Val Leu Gly Ser Ile Lys Thr Ala Lys 1610	1615	1620
Glu Leu Asp Arg Ser Asn Gln Ala Glu Tyr Asp Leu Met Val Lys 1625	1630	1635
Ala Thr Asp Lys Gly Ser Pro Pro Met Ser Glu Ile Thr Ser Val 1640	1645	1650
Arg Ile Phe Val Thr Ile Ala Asp Asn Ala Ser Pro Lys Phe Thr 1655	1660	1665
Ser Lys Glu Tyr Ser Val Glu Leu Ser Glu Thr Val Ser Ile Gly 1670	1675	1680
Ser Phe Val Gly Met Val Thr Ala His Ser Gln Ser Ser Val Val 1685	1690	1695
Tyr Glu Ile Lys Asp Gly Asn Thr Gly Asp Ala Phe Asp Ile Asn 1700	1705	1710
Pro His Ser Gly Thr Ile Ile Thr Gln Lys Ala Leu Asp Phe Glu 1715	1720	1725

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Thr Leu	Pro Ile Tyr	Thr Leu	Ile Ile Gln Gly	Thr Asn Met Ala
1730		1735		1740
Gly Leu	Ser Thr Asn Thr	Thr Val Leu Val His	Leu Gln Asp Glu	
1745		1750	1755	
Asn Asp	Asn Ala Pro Val	Phe Met Gln Ala Glu	Tyr Thr Gly Leu	
1760		1765	1770	
Ile Ser	Glu Ser Ala Ser	Ile Asn Ser Val Val	Leu Thr Asp Arg	
1775		1780	1785	
Asn Val	Pro Leu Val Ile	Arg Ala Ala Asp Ala	Asp Lys Asp Ser	
1790		1795	1800	
Asn Ala	Leu Leu Val Tyr	His Ile Val Glu Pro	Ser Val His Thr	
1805		1810	1815	
Tyr Phe	Ala Ile Asp Ser	Ser Thr Gly Ala Ile	His Thr Val Leu	
1820		1825	1830	
Ser Leu	Asp Tyr Glu Glu	Thr Ser Ile Phe His	Phe Thr Val Gln	
1835		1840	1845	
Val His	Asp Met Gly Thr	Pro Arg Leu Phe Ala	Glu Tyr Ala Ala	
1850		1855	1860	
Asn Val	Thr Val His Val	Ile Asp Ile Asn Asp	Cys Pro Pro Val	
1865		1870	1875	
Phe Ala	Lys Pro Leu Tyr	Glu Ala Ser Leu Leu	Leu Pro Thr Tyr	
1880		1885	1890	
Lys Gly	Val Lys Val Ile	Thr Val Asn Ala Thr	Asp Ala Asp Ser	
1895		1900	1905	
Ser Ala	Phe Ser Gln Leu	Ile Tyr Ser Ile Thr	Glu Gly Asn Ile	
1910		1915	1920	
Gly Glu	Lys Phe Ser Met	Asp Tyr Lys Thr Gly	Ala Leu Thr Val	
1925		1930	1935	
Gln Asn	Thr Thr Gln Leu	Arg Ser Arg Tyr Glu	Leu Thr Val Arg	
1940		1945	1950	
Ala Ser	Asp Gly Arg Phe	Ala Gly Leu Thr Ser	Val Lys Ile Asn	
1955		1960	1965	
Val Lys	Glu Ser Lys Glu	Ser His Leu Lys Phe	Thr Gln Asp Val	
1970		1975	1980	
Tyr Ser	Ala Val Val Lys	Glu Asn Ser Thr Glu	Ala Glu Thr Leu	
1985		1990	1995	
Ala Val	Ile Thr Ala Ile	Gly Asn Pro Ile Asn	Glu Pro Leu Phe	
2000		2005	2010	
Tyr His	Ile Leu Asn Pro	Asp Arg Arg Phe Lys	Ile Ser Arg Thr	
2015		2020	2025	
Ser Gly	Val Leu Ser Thr	Thr Gly Thr Pro Phe	Asp Arg Glu Gln	
2030		2035	2040	
Gln Glu	Ala Phe Asp Val	Val Val Glu Val Thr	Glu Glu His Lys	
2045		2050	2055	
Pro Ser	Ala Val Ala His	Val Val Val Lys Val	Ile Val Glu Asp	
2060		2065	2070	
Gln Asn	Asp Asn Ala Pro	Val Phe Val Asn Leu	Pro Tyr Tyr Ala	
2075		2080	2085	
Val Val	Lys Val Asp Thr	Glu Val Gly His Val	Ile Arg Tyr Val	
2090		2095	2100	
Thr Ala	Val Asp Arg Asp	Ser Gly Arg Asn Gly	Glu Val His Tyr	
2105		2110	2115	
Tyr Leu	Lys Glu His His	Glu His Phe Gln Ile	Gly Pro Leu Gly	
2120		2125	2130	

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Glu	Ile	Ser	Leu	Lys	Lys	Gln	Phe	Glu	Leu	Asp	Thr	Leu	Asn	Lys
2135						2140					2145			
Glu	Tyr	Leu	Val	Thr	Val	Val	Ala	Lys	Asp	Gly	Gly	Asn	Pro	Ala
2150						2155					2160			
Phe	Ser	Ala	Glu	Val	Ile	Val	Pro	Ile	Thr	Val	Met	Asn	Lys	Ala
2165						2170					2175			
Met	Pro	Val	Phe	Glu	Lys	Pro	Phe	Tyr	Ser	Ala	Glu	Ile	Ala	Glu
2180						2185					2190			
Ser	Ile	Gln	Val	His	Ser	Pro	Val	Val	His	Val	Gln	Ala	Asn	Ser
2195						2200					2205			
Pro	Glu	Gly	Leu	Lys	Val	Phe	Tyr	Ser	Ile	Thr	Asp	Gly	Asp	Pro
2210						2215					2220			
Phe	Ser	Gln	Phe	Thr	Ile	Asn	Phe	Asn	Thr	Gly	Val	Ile	Asn	Val
2225						2230					2235			
Ile	Ala	Pro	Leu	Asp	Phe	Glu	Ala	His	Pro	Ala	Tyr	Lys	Leu	Ser
2240						2245					2250			
Ile	Arg	Ala	Thr	Asp	Ser	Leu	Thr	Gly	Ala	His	Ala	Glu	Val	Phe
2255						2260					2265			
Val	Asp	Ile	Ile	Val	Asp	Asp	Ile	Asn	Asp	Asn	Pro	Pro	Val	Phe
2270						2275					2280			
Ala	Gln	Gln	Ser	Tyr	Ala	Val	Thr	Leu	Ser	Glu	Ala	Ser	Val	Ile
2285						2290					2295			
Gly	Thr	Ser	Val	Val	Gln	Val	Arg	Ala	Thr	Asp	Ser	Asp	Ser	Glu
2300						2305					2310			
Pro	Asn	Arg	Gly	Ile	Ser	Tyr	Gln	Met	Phe	Gly	Asn	His	Ser	Lys
2315						2320					2325			
Ser	His	Asp	His	Phe	His	Val	Asp	Ser	Ser	Thr	Gly	Leu	Ile	Ser
2330						2335					2340			
Leu	Leu	Arg	Thr	Leu	Asp	Tyr	Glu	Gln	Ser	Arg	Gln	His	Thr	Ile
2345						2350					2355			
Phe	Val	Arg	Ala	Val	Asp	Gly	Gly	Met	Pro	Thr	Leu	Ser	Ser	Asp
2360						2365					2370			
Val	Ile	Val	Thr	Val	Asp	Val	Thr	Asp	Leu	Asn	Asp	Asn	Pro	Pro
2375						2380					2385			
Leu	Phe	Glu	Gln	Gln	Ile	Tyr	Glu	Ala	Arg	Ile	Ser	Glu	His	Ala
2390						2395					2400			
Pro	His	Gly	His	Phe	Val	Thr	Cys	Val	Lys	Ala	Tyr	Asp	Ala	Asp
2405						2410					2415			
Ser	Ser	Asp	Ile	Asp	Lys	Leu	Gln	Tyr	Ser	Ile	Leu	Ser	Gly	Asn
2420						2425					2430			
Asp	His	Lys	His	Phe	Val	Ile	Asp	Ser	Ala	Thr	Gly	Ile	Ile	Thr
2435						2440					2445			
Leu	Ser	Asn	Leu	His	Arg	His	Ala	Leu	Lys	Pro	Phe	Tyr	Ser	Leu
2450						2455					2460			
Asn	Leu	Ser	Val	Ser	Asp	Gly	Val	Phe	Arg	Ser	Ser	Thr	Gln	Val
2465						2470					2475			
His	Val	Thr	Val	Ile	Gly	Gly	Asn	Leu	His	Ser	Pro	Ala	Phe	Leu
2480						2485					2490			
Gln	Asn	Glu	Tyr	Glu	Val	Glu	Leu	Ala	Glu	Asn	Ala	Pro	Leu	His
2495						2500					2505			
Thr	Leu	Val	Met	Glu	Val	Lys	Thr	Thr	Asp	Gly	Asp	Ser	Gly	Ile
2510						2515					2520			
Tyr	Gly	His	Val	Thr	Tyr	His	Ile	Val	Asn	Asp	Phe	Ala	Lys	Asp



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Glu Asp 2930	Asp Pro	Gln Gly	Gly Val	Ile Ala	Ile Leu	Ser Thr	Thr	
2935			2935		2940			
Asp Ala 2945	Asp Ser	Glu Glu	Ile Asn	Arg Gln	Val Thr	Tyr Phe	Ile	
			2950		2955			
Thr Gly 2960	Gly Asp	Pro Leu	Gly Gln	Phe Ala	Val Glu	Thr Ile	Gln	
			2965		2970			
Asn Glu 2975	Trp Lys	Val Tyr	Val Lys	Lys Pro	Leu Asp	Arg Glu	Lys	
			2980		2985			
Arg Asp 2990	Asn Tyr	Leu Leu	Thr Ile	Thr Ala	Thr Asp	Gly Thr	Phe	
			2995		3000			
Ser Ser 3005	Lys Ala	Ile Val	Glu Val	Lys Val	Leu Asp	Ala Asn	Asp	
			3010		3015			
Asn Ser 3020	Pro Val	Cys Glu	Lys Thr	Leu Tyr	Ser Asp	Thr Ile	Pro	
			3025		3030			
Glu Asp 3035	Val Leu	Pro Gly	Lys Leu	Ile Met	Gln Ile	Ser Ala	Thr	
			3040		3045			
Asp Ala 3050	Asp Ile	Arg Ser	Asn Ala	Glu Ile	Thr Tyr	Thr Leu	Leu	
			3055		3060			
Gly Ser 3065	Gly Ala	Glu Lys	Phe Lys	Leu Asn	Pro Asp	Thr Gly	Glu	
			3070		3075			
Leu Lys 3080	Thr Ser	Thr Pro	Leu Asp	Arg Glu	Glu Gln	Ala Val	Tyr	
			3085		3090			
His Leu 3095	Leu Val	Arg Ala	Thr Asp	Gly Gly	Gly Arg	Phe Cys	Gln	
			3100		3105			
Ala Ser 3110	Ile Val	Leu Thr	Leu Glu	Asp Val	Asn Asp	Asn Ala	Pro	
			3115		3120			
Glu Phe 3125	Ser Ala	Asp Pro	Tyr Ala	Ile Thr	Val Phe	Glu Asn	Thr	
			3130		3135			
Glu Pro 3140	Gly Thr	Leu Leu	Thr Arg	Val Gln	Ala Thr	Asp Ala	Asp	
			3145		3150			
Ala Gly 3155	Leu Asn	Arg Lys	Ile Leu	Tyr Ser	Leu Ile	Asp Ser	Ala	
			3160		3165			
Asp Gly 3170	Gln Phe	Ser Ile	Asn Glu	Leu Ser	Gly Ile	Ile Gln	Leu	
			3175		3180			
Glu Lys 3185	Pro Leu	Asp Arg	Glu Leu	Gln Ala	Val Tyr	Thr Leu	Ser	
			3190		3195			
Leu Lys 3200	Ala Val	Asp Gln	Gly Leu	Pro Arg	Arg Leu	Thr Ala	Thr	
			3205		3210			
Gly Thr 3215	Val Ile	Val Ser	Val Leu	Asp Ile	Asn Asp	Asn Pro	Pro	
			3220		3225			
Val Phe 3230	Glu Tyr	Arg Glu	Tyr Gly	Ala Thr	Val Ser	Glu Asp	Ile	
			3235		3240			
Leu Val 3245	Gly Thr	Glu Val	Leu Gln	Val Tyr	Ala Ala	Ser Arg	Asp	
			3250		3255			
Ile Glu 3260	Ala Asn	Ala Glu	Ile Thr	Tyr Ser	Ile Ile	Ser Gly	Asn	
			3265		3270			
Glu His 3275	Gly Lys	Phe Ser	Ile Asp	Ser Lys	Thr Gly	Ala Val	Phe	
			3280		3285			
Ile Ile 3290	Glu Asn	Leu Asp	Tyr Glu	Ser Ser	His Glu	Tyr Tyr	Leu	
			3295		3300			
Thr Val 3305	Glu Ala	Thr Asp	Gly Gly	Thr Pro	Ser Leu	Ser Asp	Val	
			3310		3315			
Ala Thr 3320	Val Asn	Val Asn	Val Thr	Asp Ile	Asn Asp	Asn Thr	Pro	
			3325		3330			

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Val Phe Ser Gln Asp Thr Tyr Thr Thr Val Ile Ser Glu Asp Ala 3335 3340 3345
Val Leu Glu Gln Ser Val Ile Thr Val Met Ala Asp Asp Ala Asp 3350 3355 3360
Gly Pro Ser Asn Ser His Ile His Tyr Ser Ile Ile Asp Gly Asn 3365 3370 3375
Gln Gly Ser Ser Phe Thr Ile Asp Pro Val Arg Gly Glu Val Lys 3380 3385 3390
Val Thr Lys Leu Leu Asp Arg Glu Thr Ile Ser Gly Tyr Thr Leu 3395 3400 3405
Thr Val Gln Ala Ser Asp Asn Gly Ser Pro Pro Arg Val Asn Thr 3410 3415 3420
Thr Thr Val Asn Ile Asp Val Ser Asp Val Asn Asp Asn Ala Pro 3425 3430 3435
Val Phe Ser Arg Gly Asn Tyr Ser Val Ile Ile Gln Glu Asn Lys 3440 3445 3450
Pro Val Gly Phe Ser Val Leu Gln Leu Val Val Thr Asp Glu Asp 3455 3460 3465
Ser Ser His Asn Gly Pro Pro Phe Phe Phe Thr Ile Val Thr Gly 3470 3475 3480
Asn Asp Glu Lys Ala Phe Glu Val Asn Pro Gln Gly Val Leu Leu 3485 3490 3495
Thr Ser Ser Ala Ile Lys Arg Lys Glu Lys Asp His Tyr Leu Leu 3500 3505 3510
Gln Val Lys Val Ala Asp Asn Gly Lys Pro Gln Leu Ser Ser Leu 3515 3520 3525
Thr Tyr Ile Asp Ile Arg Val Ile Glu Glu Ser Ile Tyr Pro Pro 3530 3535 3540
Ala Ile Leu Pro Leu Glu Ile Phe Ile Thr Ser Ser Gly Glu Glu 3545 3550 3555
Tyr Ser Gly Gly Val Ile Gly Lys Ile His Ala Thr Asp Gln Asp 3560 3565 3570
Val Tyr Asp Thr Leu Thr Tyr Ser Leu Asp Pro Gln Met Asp Asn 3575 3580 3585
Leu Phe Ser Val Ser Ser Thr Gly Gly Lys Leu Ile Ala His Lys 3590 3595 3600
Lys Leu Asp Ile Gly Gln Tyr Leu Leu Asn Val Ser Val Thr Asp 3605 3610 3615
Gly Lys Phe Thr Thr Val Ala Asp Ile Thr Val His Ile Arg Gln 3620 3625 3630
Val Thr Gln Glu Met Leu Asn His Thr Ile Ala Ile Arg Phe Ala 3635 3640 3645
Asn Leu Thr Pro Glu Glu Phe Val Gly Asp Tyr Trp Arg Asn Phe 3650 3655 3660
Gln Arg Ala Leu Arg Asn Ile Leu Gly Val Arg Arg Asn Asp Ile 3665 3670 3675
Gln Ile Val Ser Leu Gln Ser Ser Glu Pro His Pro His Leu Asp 3680 3685 3690
Val Leu Leu Phe Val Glu Lys Pro Gly Ser Ala Gln Ile Ser Thr 3695 3700 3705
Lys Gln Leu Leu His Lys Ile Asn Ser Ser Val Thr Asp Ile Glu 3710 3715 3720
Glu Ile Ile Gly Val Arg Ile Leu Asn Val Phe Gln Lys Leu Cys





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Asp	Glu	Cys	Ser	Gly	Asn	Pro	Cys	Leu	His	Gly	Ala	Leu	Cys	Glu
4130						4135					4140			
Asn	Thr	His	Gly	Ser	Tyr	His	Cys	Asn	Cys	Ser	His	Glu	Tyr	Arg
4145						4150					4155			
Gly	Arg	His	Cys	Glu	Asp	Ala	Ala	Pro	Asn	Gln	Tyr	Val	Ser	Thr
4160						4165					4170			
Pro	Trp	Asn	Ile	Gly	Leu	Ala	Glu	Gly	Ile	Gly	Ile	Val	Val	Phe
4175						4180					4185			
Val	Ala	Gly	Ile	Phe	Leu	Leu	Val	Val	Val	Phe	Val	Leu	Cys	Arg
4190						4195					4200			
Lys	Met	Ile	Ser	Arg	Lys	Lys	Lys	His	Gln	Ala	Glu	Pro	Lys	Asp
4205						4210					4215			
Lys	His	Leu	Gly	Pro	Ala	Thr	Ala	Phe	Leu	Gln	Arg	Pro	Tyr	Phe
4220						4225					4230			
Asp	Ser	Lys	Leu	Asn	Lys	Asn	Ile	Tyr	Ser	Asp	Ile	Pro	Pro	Gln
4235						4240					4245			
Val	Pro	Val	Arg	Pro	Ile	Ser	Tyr	Thr	Pro	Ser	Ile	Pro	Ser	Asp
4250						4255					4260			
Ser	Arg	Asn	Asn	Leu	Asp	Arg	Asn	Ser	Phe	Glu	Gly	Ser	Ala	Ile
4265						4270					4275			
Pro	Glu	His	Pro	Glu	Phe	Ser	Thr	Phe	Asn	Pro	Glu	Ser	Val	His
4280						4285					4290			
Gly	His	Arg	Lys	Ala	Val	Ala	Val	Cys	Ser	Val	Ala	Pro	Asn	Leu
4295						4300					4305			
Pro	Pro	Pro	Pro	Pro	Ser	Asn	Ser	Pro	Ser	Asp	Ser	Asp	Ser	Ile
4310						4315					4320			
Gln	Lys	Pro	Ser	Trp	Asp	Phe	Asp	Tyr	Asp	Thr	Lys	Val	Val	Asp
4325						4330					4335			
Leu	Asp	Pro	Cys	Leu	Ser	Lys	Lys	Pro	Leu	Glu	Glu	Lys	Pro	Ser
4340						4345					4350			
Gln	Pro	Tyr	Ser	Ala	Arg	Glu	Ser	Leu	Ser	Glu	Val	Gln	Ser	Leu
4355						4360					4365			
Ser	Ser	Phe	Gln	Ser	Glu	Ser	Cys	Asp	Asp	Asn	Gly	Tyr	His	Trp
4370						4375					4380			
Asp	Thr	Ser	Asp	Trp	Met	Pro	Ser	Val	Pro	Leu	Pro	Asp	Ile	Gln
4385						4390					4395			
Glu	Phe	Pro	Asn	Tyr	Glu	Val	Ile	Asp	Glu	Gln	Thr	Pro	Leu	Tyr
4400						4405					4410			
Ser	Ala	Asp	Pro	Asn	Ala	Ile	Asp	Thr	Asp	Tyr	Tyr	Pro	Gly	Gly
4415						4420					4425			
Tyr	Asp	Ile	Glu	Ser	Asp	Phe	Pro	Pro	Pro	Pro	Glu	Asp	Phe	Pro
4430						4435					4440			
Ala	Ala	Asp	Glu	Leu	Pro	Pro	Leu	Pro	Pro	Glu	Phe	Ser	Asn	Gln
4445						4450					4455			
Phe	Glu	Ser	Ile	His	Pro	Pro	Arg	Asp	Met	Pro	Ala	Ala	Gly	Ser
4460						4465					4470			
Leu	Gly	Ser	Ser	Ser	Arg	Asn	Arg	Gln	Arg	Phe	Asn	Leu	Asn	Gln
4475						4480					4485			
Tyr	Leu	Pro	Asn	Phe	Tyr	Pro	Leu	Asp	Met	Ser	Glu	Pro	Gln	Thr
4490						4495					4500			
Lys	Gly	Thr	Gly	Glu	Asn	Ser	Thr	Cys	Arg	Glu	Pro	His	Ala	Pro
4505						4510					4515			
Tyr	Pro	Pro	Gly	Tyr	Gln	Arg	His	Phe	Glu	Ala	Pro	Ala	Val	Glu
4520						4525					4530			

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Ser Met Pro Met Ser Val Tyr Ala Ser Thr Ala Ser Cys Ser Asp  
 4535 4540 4545

Val Ser Ala Cys Cys Glu Val Glu Ser Glu Val Met Met Ser Asp  
 4550 4555 4560

Tyr Glu Ser Gly Asp Asp Gly His Phe Glu Glu Val Thr Ile Pro  
 4565 4570 4575

Pro Leu Asp Ser Gln Gln His Thr Glu Val  
 4580 4585

<210> SEQ ID NO 3  
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ccccttccaa ctctccctca 20

<210> SEQ ID NO 4  
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caggctctcc cgggcactgt 20

<210> SEQ ID NO 5  
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 <220> FEATURE:  
 <223> OTHER INFORMATION: Mouse Fat1 siRNA template

<400> SEQUENCE: 5

ggaccgaagt caccaagta 19

<210> SEQ ID NO 6  
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 <213> ORGANISM: artificial  
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<400> SEQUENCE: 6

gcgacgcatt taacattaa 19

<210> SEQ ID NO 7  
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 <223> OTHER INFORMATION: Mouse Fat1 siRNA template 3

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gcatgacact ttaaataaa 19

<210> SEQ ID NO 8  
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 <213> ORGANISM: artificial  
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 <223> OTHER INFORMATION: Mouse Fat1 siRNA template

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19

<210> SEQ ID NO 9

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<213> ORGANISM: artificial

<220> FEATURE:

<223> OTHER INFORMATION: control siRNA. Scrambled derivative of the  
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gtaaccataa acaggcatt

19

<210> SEQ ID NO 10

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: artificial

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<223> OTHER INFORMATION: control siRNA. Mismatched derivative of the  
7296 sequence.

<400> SEQUENCE: 10

gtctgataat gcgcataaa

19

<210> SEQ ID NO 11

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: artificial

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<223> OTHER INFORMATION: Mouse Fat1IC RT-PCR forward primer

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30

<210> SEQ ID NO 12

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: artificial

<220> FEATURE:

<223> OTHER INFORMATION: Mouse Fat1IC RT-PCR reverse primer

<400> SEQUENCE: 12

tctagacact tccgtatgct gctggga

27

<210> SEQ ID NO 13

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: artificial

<220> FEATURE:

<223> OTHER INFORMATION: forward primer for generating Fat 4189-4587

<400> SEQUENCE: 13

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29

<210> SEQ ID NO 14

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: artificial

<220> FEATURE:

<223> OTHER INFORMATION: forward primer for generating Fat14201-4587

<400> SEQUENCE: 14

ccatgggccca ggctgaacct gaagacaaac

30

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What is claimed is:

1. A method of inhibiting vascular smooth muscle cell (VSMC) proliferation in a patient having a bypass surgery vascular repair or an angioplasty vascular repair, the method comprising administering to the patient having the vascular repair an amount of a fusion protein consisting essentially of the sequence of residues 4202-4588 of SEQ ID NO:2 fused to the amino acid sequence of an extracellular domain and trans-membrane region of an interleukin 2 receptor  $\alpha$ -chain which localizes to a cell membrane effective to inhibit (VSMC) proliferation.

2. The method of claim 1, wherein the vascular repair is an angioplasty vascular repair.

3. The method of claim 1, wherein the patient is at risk of restenosis.

4. The method of claim 1, wherein the vascular repair is a bypass surgery vascular repair.

5. The method of claim 1, wherein the vascular repair is of a coronary artery.

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